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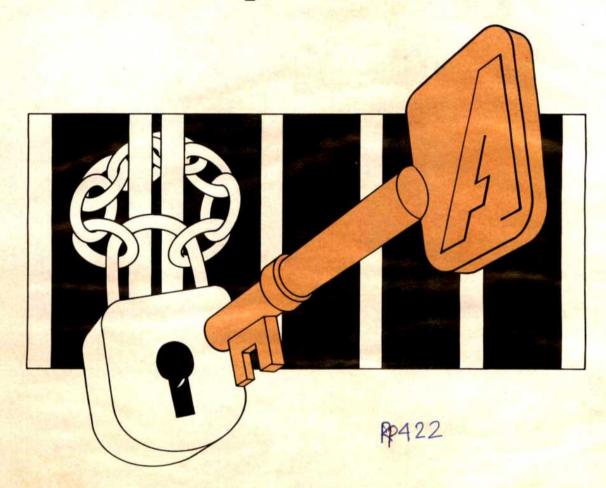
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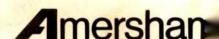
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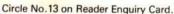


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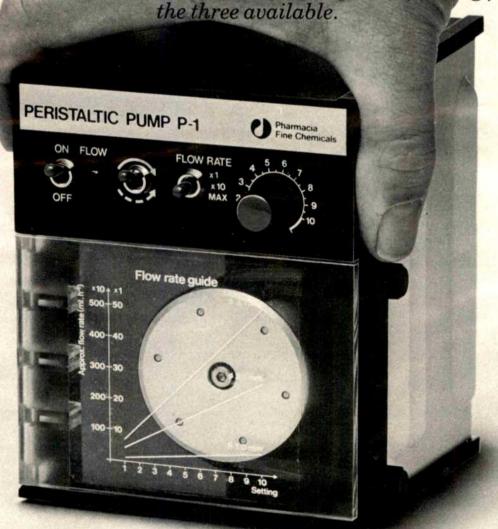
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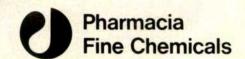
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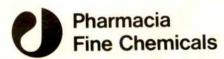
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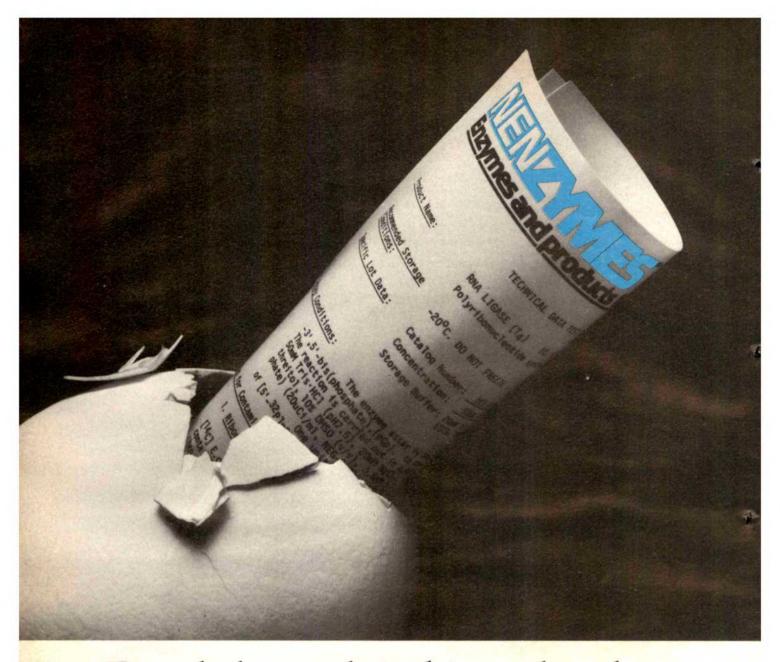
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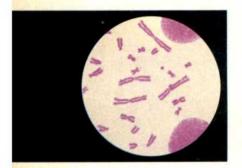
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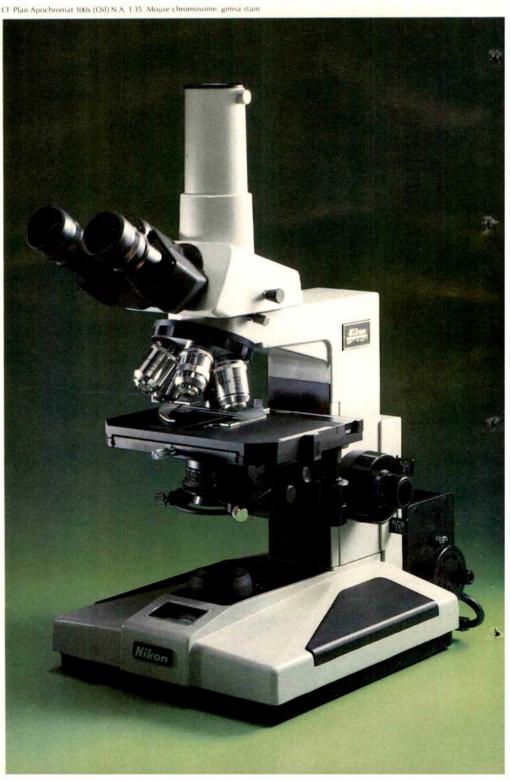
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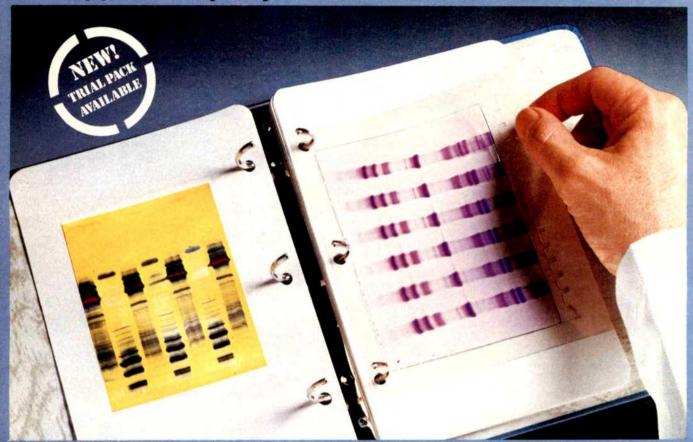
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Nature* (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Journals Ltd and includes the Directory of Biologicals (mailed in December) and Annual Index (mailed in February). Annual subscription for USA and Canada US \$198.50 (for subscription prices elsewhere, see next page). Orders (with remittance) and change of address labels to: Macmillan Journals Ltd, Brunel Rd, Basingstoke RG21 2XS, UK. Second class postage paid at New York, NY 10010 and additional mailing offices. US Postmaster send form 3579 to: Nature, 15 East 26 Street, New York, NY 10010. ◎ 1982 Macmillan Journals Ltd.



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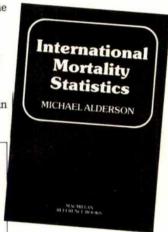
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Nature B ISSN 0028-0836 Registered as a newspaper at the British Post Office ©1982 Macmillan Journals Ltd

Vol. 296 No. 5860 29 April 1982

nature

4 March 1982

Universities, profit and research

The presidents of five major research universities will meet privately later in the month to work out guidelines for commercializing research. They cannot be allowed the last word.

Dr Donald Kennedy, president of Stanford University, seems to have been granted his wish, frequently expressed a year ago, for a high-level conference on the problems of the relationships between universities and the commercial interests in recombinant DNA research. Later this month (and not far on the Californian coast from Asilomar), he and four other university presidents, (California, Caltech, Harvard and MIT) hope to assemble with select teams of their senior colleagues and industrial partners to hammer out guidelines for the proper conduct of joint commercial ventures. Although this occasion cannot match that in 1973 at which a relatively unstructured gathering of molecular biologists agreed, off their own bat, that there should be guidelines to regulate the conduct of recombinant DNA research. there is nothing reprehensible about what is now proposed. The five universities concerned are those which have been most intimately caught up with the commercialization of recombinant DNA research. What more reasonable than that they should arrange privately to meet to talk about their problems and to agree, if possible, to some common approach to the problems that have not yet arisen? There is no reason why they should be deflected from this sensible — and overdue — exchange of ideas by the wish of others, congressmen or other universities, to join the action.

Equally, however, it would be wrong for those planning to meet three weeks from now to think that what they agree among themselves about the proper relationship between universities and outside commercial interests will apply to other universities and other commercial interests. Indeed, the question is bound to arise whether any guidelines arrived at can be successfully applied even within the five participating universities — one of which, the University of California, is already in some difficulty with the state authorities on the customary exemption of academics who are also state employees from the standard constraints on conflicts of interest. This month's private meeting is bound to sharpen questions about the propriety of what the university intends. But there are also limits to the extent to which even the private universities can write their own tickets into a brave newcommercial future. Already, as the affairs of the University of California's Davis campus have shown (see p.6), the interests of graduate students and those who teach them are not identical and, for a research university, the contentment of the graduate students takes precedence over that of the faculty. More generally but more importantly, the universities, especially the best among them, are public institutions embedded in a society which is perfectly entitled to views on how they should be run. And there are tax-exemption rules to give force to public opinion. This month's private conference could do a useful job, especially if it follows the sensible course of making public its conclusions. But it cannot be the last word.

What, in the circumstances, should Dr Kennedy and his colleagues talk about? Their first task must be to preserve the integrity of the institution of the university. Since the first excitement about the commercial potential of biotechnology five years or so ago, most universities have been in two minds about the roles that they should play. The prospect that they might, as a consequence of the cleverness of individual members of their faculties, be partly immunized from external financial pressures, has naturally been attractive. And government (not merely in the

United States) has been urging that universities should find other than public sources of financial support. But, dare it be said, not all biotechnology and not all biotechnologists automatically satisfy the criteria of excellence which universities, and especially the good universities, seek to apply to their affairs. How, then, to channel an institution's enthusiasm into support for some activity that will make a little money while giving proper academic attention to other activities that bring no profit? The ideal is that university faculties should be even more zealously encouraged than at present to decide among themselves between the good and the less good. The ideal is simple, but its practice will be hard.

Problems concerning people are likely to be even more important. It is essential that means should be found of making sure that universities cannot be fairly charged with misusing their students, perhaps by allowing faculty members to turn some piece of graduate research to commercial use. Similarly, because the effectiveness of universities requires a certain seemliness within the whole body of faculty and students, it is important that no single member of such a community should be thought capable of turning his academic position into unreasonable riches. And however intellectually absorbing may be the task of turning a bright idea into some commercial innovation, there must be a limit to the extent to which a university can let full-time members of its faculty function as entrepreneurs while remaining in the fold. Stanford has already made some headway with its internally promulgated rules for deciding how financial benefits from commercial enterprises should be shared between individuals. departments and the university. What is needed, now, is not so much a wider adoption of some standard formula as a better understanding of how such arrangements are working. Although biotechnology still seems the most conspicuous opportunity for turning academic work to profit, there are other betterestablished fields (engineering, for example) from which much can be learned. The trouble is that a clear understanding of this point will require an investigation, not just a two-day meeting.

British nuclear weapons

New nuclear submarines may be the best buy; talking to the French would be safer.

The British government's present dilemma about nuclear weapons policy is at least familiar. For at least the past twenty years, there has been continuing doubt about the most suitable means of preparing to deliver the modest British stock of nuclear explosives. It all seems so much more complicated than in the faroff 1950s when Britain, having become a nuclear power in a fit of absence of mind, was able to keep up with the superpowers with the help of home-built aircraft. Since then, it seems, there has been nothing but trouble. Knowing that a nuclear striking force is no more credible than the means by which explosives can be delivered to intended targets, the British government embarked on the development of intermediate-range missiles (remember Bluestreak?) and contour-following aircraft (the TSR2). But the pace turned out to be too demanding. This is why, in 1962, the British government leapt at the deal offered by President John F. Kennedy and took out an option first on the weapon called Skybolt (an early air-launched cruise missile that was promptly

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cancelled) and then on *Polaris* missiles carried in British-built submarines. Now, with both the submarines and the rockets which they carry nearing the end of their useful life, the British government is doing what must now seem natural, and is planning to follow the United States in replacing *Polaris* by its more powerful (and accurate) successor, *Trident*.

The whole sorry tale should be an object lesson, and a warning, to other states flirting with the notion that they, too, might become second or third-rank nuclear powers. It should also help to moderate some of the more alarmist notions of how easily nuclear weapons might proliferate. For nobody believes that the cost of this still incomplete system will be contained within the £6,000 million (spread over fifteen years) that the Ministry of Defence is talking about. The argument that the total cost will be. in any case, only three per cent of the British defence budget over the fifteen-year construction period is hardly what matters — the cost works out at seven per cent of the likely equipment budget over that period. This prospect chimes ironically with the British government's decision to put several relatively new items of defence equipment on the secondhand market — the aircraft carrier Invincible is for example being sold at cost to Australia, while the Indian government is hoping to pick up an Antarctic research vessel (see Nature 25 February, p.640). Yet the cost of the British weapons programme, blessed by the Bermuda agreement, is undoubtedly far less than would be entailed by complete independence — as French taxpayers should be well aware.

The cost of being a serious nuclear power, even a small one, is onerous. The British government's domestic critics (who, on this issue, include a great many of its natural supporters and even one field-marshal) have made several telling points. Conventional defences will be weakened just when they need to be reinforced. The cost of Trident could better be spent on more creative projects. And now, for the first time since 1958, there is a risk that bi-partisan agreement that Britain should have some kind of nuclear defence force will disappear. No doubt each of these complaints carries some weight. The surprise, however, is that the argument between the British government and its critics has been confined to more or less tactical questions. Would cruise missiles be a better buy than Trident? (The answer is maybe.) Would the same expenditure on conventional forces contribute more to the defence of Western Europe? (Almost certainly yes.) The curious aspect of the row about Trident, however, is that so little is being done to clarify the reasons for and against the retention of an independent nuclear force of any kind.

In the 1950s, it was different. The decision then to develop an independent nuclear force stimulated a vigorous and informative argument. Serious opinion was split three ways. At one extreme were the unilateral disarmers, whose residual legatees seem now more anxious to prevent the siting of United States nuclear weapons in Britain than to re-examine British nuclear policy as a whole. Then, in the 1950s, what might be called liberal military opinion argued that British nuclear weapons might have a useful role in an escalating European conflict. The government view, which prevailed and has since stuck, was that an independent British nuclear force would help to cement the commitment of the United States to its European alliances, for it would be easier to stand aside from a conventional than from a nuclear conflict. Diplomatically, of course, the argument was never put so directly, for that would have invited the United States Congress to refuse to sanction the technical assistance with which the British nuclear force was built. Now, for what it is worth, the British case appears subtly to have changed. Last weekend, the Secretary of State for Defence, Mr John Nott, was arguing in a television interview that the Trident force, like and with its French equivalent, would come into its own if the pattern of alliances in Western Europe changed, leaving both France and Britain to look after their own defence.

This argument is essentially the same as the Gallois doctrine in the 1950s to justify the French nuclear force. To defend itself with nuclear weapons, a state merely has to equip itself with the means of inflicting damage on a potential enemy proportional to its own value as a prize. The argument is persuasive, but it conceals a serious danger — its universal applicability. If French and British

nuclear forces are justified in this way, why not Swiss or Swedish? The practical answer may be that at this late stage in the development of nuclear technology, the entry fee would be too high. But nobody can be sure. And if the British government is indeed edging round to planning for a world in which the pattern of European alliances may have radically changed, should it not now be talking to the French government, which must share the long-term interest that Europe should not become a collection of supposedly independent nuclear powers. And, by the same test, there is much to be said for going as slowly as possible with *Trident*, for the sake of all the money that may then be saved.

Chemical inspections

British proposals for verifying a chemical weapons treaty should be taken seriously.

The abiding trouble with arms control is that there is so much talk about it. Even if the apparently endless negotiations in Vienna on Mutually Balanced Force Reductions are forgotten (which is probably the most appropriate course), there are at present no fewer than four settings for serious discussions on the subject: the European Security Conference in Madrid (made necessary by the Helsinki agreements), the superpower talks in Geneva on European nuclear weapons, the standing Committee on Disarmament now also meeting in Geneva and the labyrinthine preparations for the United Nations session on disarmament, planned for June. Of these, however, only the two Geneva meetings are for the time being serious, and the United States and the Soviet Union have so far kept to their agreement not to broadcast news of what is going on. This is why it is refreshing that something sensible is happening at the other meeting in Geneva, that of the Committee on Disarmament.

The British government, often pilloried (as in the preceding account of its nuclear weapons policy), last week startled the Committee on Disarmament by tackling head-on one of the most difficult of the technical problems impeding the negotiation of a treaty to get rid of chemical weapons — that of verification. The problem is by now all too familiar. The agents potentially usable as chemical weapons can be made in manufacturing plants which are indistinguishable from those outside from the used for making pacific chemicals of various kinds. Although chemical munitions plants are by convention usually sited far away from civilian populations, there is no reason why their isolation should be ostentatious. The result is that remote surveillance, from satellites for example, cannot reliably indicate whether a state that has signed a treaty to dismantle its stocks of chemical weapons and to refrain from making more is actually keeping to the rules.

The British view, put forward last week, is that a sufficient degree of assurance against violations of a treaty could be provided by a judicious use of an expert committee, the monitoring of the flow of chemical raw materials through chemical processes, a complaints procedure and the judicious use of on-site inspection. Nobody can at this stage guess how the proposal will be received. The Soviet Union is notoriously sensitive about proposals for on-site inspection, but is not the only manufacturer of chemicals that is also touchy about its sovereignty. Sooner or later, however, on-site inspection is going to have to be a component of arms control agreements — and was, two years ago, accepted by all the nuclear powers to be a necessary part of the test-ban treaty then in draft. So the British government deserves encouragement for having raised the issue again. And it deserves some credit for trying to breathe life into an arms control project that has been too long forgotten since the superpower talks on the subject broke down in 1980. For this is eminently a field in which all potential parties to future conflicts would gain from an agreement not to manufacture weapons whose military value is as much in doubt as that of chemical weapons, whose production, maintenance and occasional disposal is a constant threat to the domestic population and whose agreed abolition would rid the world of a great nuisance and a needless cost.

Clouds on biotechnology horizon

Companies run short of sales and capital

Washington

The US biotechnology industry is coming down to earth with a bump, as various companies begin to experience difficulty in raising the capital needed to finance ambitious expansion plans.

Last week, for example, Bethesda Research Laboratories (BRL), one of the more successful of the many small biotechnology companies to have been set up within the past few years, announced that it was cutting back its workforce from 450 to just under 300 because of cash-flow problems, and was re-thinking plans for moving into larger facilities.

Only six months ago, the company was talking of a public offer of its stock, seeking to raise \$40 million to finance expansion over the next two or three years.

Other companies are experiencing the same problem, although none quite so dramatically. San Francisco-based Genentech, for example, is expected to have to think hard about how it is going to raise the new capital it will soon need for future expansion, although it said last week that at present the company was growing at its projected rate.

So far, out of more than 150 companies set up in the past few years, only one — Applied Genetics of Boston — is known to have folded. In most cases, according to Wall Street analysts, the cutbacks in growth being reported reflect a shake-out that had long been expected, partly a reaction against a period of over-expectation.

They point out that in the case of BRL, for example, the company is retaining most of its scientific personnel, particularly in the area of restriction enzymes for which it has established a worldwide reputation. The cuts are being made in more peripheral areas in order to maintain a healthy core.

The problems being experienced by the biotechnology companies are caused less by the current recession than by the fact that it is now much more difficult to raise new equity than it was even six months ago.

After the dramatic sale of stock in Genentech exactly a year ago, when shares rose from \$35 to \$80 on the first day of trading, shares have now stabilized around the original figure. Shares in Cetus, which went public with a far bigger proportion of its equity six months later, have slipped from \$25 to around \$13. The latest public newcomer, Collaborative Research of Waltham, Massachusetts, also received a relatively cool reaction when it went public last month.

Investment analysts claim these figures reflect a new realism in the market, which recognizes that although genetic engineering techniques still offer a large commercial potential, there is still some way to go before anyone starts reporting a substantial profit. Two weeks ago, for example, Genentech reported that profits for 1981 were double those of 1980, but at \$300,000 these compare unfavourably with a revenue of \$21.3 million.

In the case of BRL, since the company is still private and therefore does not have access to large amounts of capital, it has had to rely more heavily on the sale of laboratory products, which last year generated an income of over \$10 million. This has made the company particularly sensitive to any fluctuations in the market, contributing directly to the decision to "restructure" its activities.

BRL is divided into four divisions covering research products, instrumentation, genetics research and molecular diagnostics. Staffing cuts are being distributed across all four divisions, and although ten PhDs were among those who had received their notice, the company says that in general the cuts had fallen less heavily on scientists (who make up about one-sixth of employees) than on other staff.

The cuts mark the end of a period of

"NEVER MIND-IF THINGS LOOK UP, WE'LL CLONE A FEW MORE."



spectacular growth for BRL. Formed by Stephen Turner, who previously worked with Becton-Dickinson, with two other employees in 1976 and based close to the National Institutes of Health which have been among its most important clients for restriction enzymes, the company had sales of \$350,000 in 1977, \$1 million in 1978 and \$5 million in 1980.

Asked in an newspaper interview last September how he viewed the future prospects for his company, Mr Turner admitted that the market for his products was rapidly filling up as the expansion of new genetic engineering laboratories levelled off. "I was taught long ago that the goal of business planning is survival, not dominance", he said.

David Dickson

Cohen-Boyer patent extended

Washington

The US Patent Office last week informed Stanford University and the University of California, San Francisco (UCSF) that it has accepted their claims for a patent on any microorganism which is made using the genetic engineering techniques developed by Dr Stanley Cohen of Stanford and Dr Herbert Boyer of UCSF.

The techniques themselves were granted a patent just over a year ago. The patent was offered for licensing last summer, and last week Mr Andrew Barnes of Stanford's Office of Technology Licensing said 73 companies have so far agreed to pay the \$10,000 annual licence fee (five times which is credited against any future royalty payments).

Initially, applications for patents on both the techniques and the products of the techniques were filed simultaneously. However, in the light of the Patent Office's subsequent challenge to the legality of patenting living organisms — eventually resolved by the US Supreme Court, which agreed that they could be patented — the application was split into two parts.

Of the two, Mr Barnes said that the process patent already granted was considered by the universities to be the more significant. He added, however, that

the product patent, which it had been generally anticipated that the universities would receive, could strengthen their hand in any future legal proceedings.

For example, if the process patent alone had been allowed, foreign companies might have been tempted to import into the United States products made in countries where the techniques had not been patented, and the universities would have had to challenge this under a slightly ambiguous ruling from the International Trade Commission. Now that the product patent, expected to be formally granted in a few weeks, has been allowed, it will be a legal challenge to such imports.

The new patent will cover any recombinant plasmid made using the techniques developed by Dr Cohen and Dr Boyer, provided that the vector is compatible with the cellular host organism. The universities have also been allowed a patent on the transformant itself, providing this is a unicellular organism, such as a bacterium or a protozoan; human and plant cells, however, would not be covered.

Rights to the use of the products are already included in the licensing agreement which Stanford and UCSF have been negotiating with interested companies.

David Dickson

Pharmaceutical research

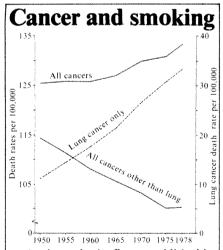
New tenants

The Swiss pharmaceutical company Sandoz has decided to set up a new research institute in the neurosciences at University College, part of the University of London. According to Mr E.J. Fullagar, director of Sandoz's UK operations, the new institute reflects the company's belief in the quality of British researchers — it hopes to recruit almost all its staff in Britain — and in the continuance of top quality British research. But the institute may help to persuade the British regulatory authorities to view more favourably applications for price increases on Sandoz's products.

The new institute, the first that Sandoz has set up on a university campus, is an experiment. According to Mr Fullagar, the venture will be no less expensive than building an institute on Sandoz's own premises. The hope is that spin-off from close links with the college will more than pay for any extra costs incurred.

The company chose to site its institute at University College partly because the two have collaborated successfully in the past on clinical studies and partly because the college's research in the neurosciences matches its own interests. The new institute will be chiefly concerned with the role of neurotransmitters and neuromodulators in the nervous system.

Although the institute will be entirely independent of the college, employing



As shown in the figure, published in "The Health Consequences of Smoking: Cancer 1982" (US Department of Health and Human Services), during the period 1950 to 1978, the overall cancer death rate increased. This trend results from a remarkable increase in lung cancer death rates. However, if lung cancer deaths are excluded, the overall cancer mortality rate has fallen. This is largely a result of improved diagnosis, treatment and survival times for cancer sites not associated with cigarette smoking, such as prostatic, colorectal and breast cancers, while lung cancer survival remains dismal.

about 60 of its own research staff, the plan is that there should be a close relationship between the research staffs and that the college might look to some institute staff for teaching.

The college, which although relatively well endowed is nevertheless suffering from university cutbacks, will benefit financially. Although Sandoz's rent is only nominal, the company will pay the college a £2.5 million premium and thereafter pay for overheads and services such as library and catering facilities. The money will help the college out of some of its difficulties, in particular those of completing capital projects abandoned because of cuts in its grants from the University Grants Committee. Thus work begun on a new chemistry building in the early 1970s will now be completed. Sandoz will refurbish the building vacated when the chemistry department moves into the new building to be used by the new institute.

Sandoz estimates that it will spend £5 million on setting up the institute, divided equally between payments to the college, the costs of refurbishing and the cost of equipping laboratories. The laboratories will not be ready before September 1983 at the earliest. Hence, according to Mr Fullagar, it is still too early to describe the research programme in detail, or to say whether the college and institute will cooperate on joint programmes and, if so, how. Patents taken out on the results of research supported entirely by the institute will, however, belong to Sandoz. But where research has been jointly supported, the college's lawyers will advise.

Sandoz, therefore, seems cheerful about the long-term prospects of British university research. Its view, however, is not shared by Biogen, the Swiss-based pharmaceutical company, which has chosen not to site its third laboratory in Britain on the grounds that the university cuts may irreparably damage British biotechnological research.

Biogen plans to set up a new laboratory along the lines of those at Cambridge, Massachusetts, and Geneva. The idea is that the laboratory should be separate from but near to a university department with considerable expertise in recombinant DNA research. The company says that it cannot justify setting up a laboratory near a suitable British university because no department is guaranteed to escape the ill effects of the university cuts. So it is looking elsewhere, in particular in Belgium and West Germany.

The new laboratory is intended to complement the work of Biogen's existing laboratories and to allow the company to spread its research between institutions employing no more than 75 research staff. According to Walter Gilbert, Biogen's chief executive, Biogen will nevertheless consider employing British biotechnologists of suitable calibre who apply for posts in any of its laboratories.

Judy Redfearn

Pesticide data

Row on access

Washington

The latest move in the lengthy battle over access to scientific data on the medical effects of pesticides came last month with the US Environmental Protection Agency (EPA) asking Congress for authority to write its own rules for the release of such information. Members of the House of Representatives' Agriculture Committee have tried to find a compromise between public interest groups demanding open access to the data submitted to the agency and pesticide manufacturers, who argue that the unrestricted release of such information would benefit their commercial competitors.

The manufacturers have been pushing for changes in the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), first passed by Congress in 1972, which provides any individual with access under the Freedom of Information Act to scientific and medical data submitted to EPA as part of the regulatory process of registering a new chemical. Attempts at revision are being fought by a coalition of health groups, conservationists and labour unions which argues that the demands being made by industry would make it impossible, for example, for research scientists to communicate openly the results of their analysis of the data.

In its zeal to respond to public protests about the potential health hazards of new pesticides, Congress passed in 1978 stiff new amendments to FIFRA. These included a specific clause saying that data on the medical effects of a pesticide were not to be treated as a trade secret, and were not therefore exempt from freedom-of-information requests.

Since then, manufacturers working primarily through their Washington-based lobbying organization, the National Agriculture Chemicals Association (NACA), have tried to challenge the clause several times in court. The companies claim that the data produced in their research laboratories are private, and that the government's requirement that they be handed out on demand is a violation of constitutional rights.

So far, the courts have upheld the validity of the new law. The manufacturers have responded by turning their attention first to Congress, in the hope of having the law revised, and more recently to EPA with the prospect of receiving a more sympathetic hearing.

Last summer Mr George Brown, previously a prominent member of the House Science and Technology Committee and now chairman of the research subcommittee of the House Agriculture Committee, introduced a new bill that would limit the conditions under which the data could be released. These revisions would make the data available only to approved

individuals, who would have to take notes from reports made available in EPA reading rooms. Labour union representatives and people working in the health services would be able to borrow complete reports, but would not be allowed to divulge the contents to others. No employee of another chemical manufacturing company would be allowed access to the data; and nobody would be allowed to make a complete transcript of the research reports.

Members of the subcommittee staff argue that such provisions, provide a compromise between industry's desire to protect confidential research data, and the public's right to information on the risks and benefits of chemicals used for crop protection. Both parties in the dispute disagree.

The manufacturers were initially enthusiastic about the changes proposed by Mr Brown, but their support has evaporated as the amendments have been revised to take into account some of the objections of those claiming to be defending the public interest.

Environmentalists and labour union groups are seizing this "window of opportunity" between the failure of the last industry challenge and the eventual tightening up of administrative requirements to get as much information on existing pesticides out of EPA as they can. EPA administrator Anne Gorsuch informed a coalition of such groups that the agency intended to respond to demands for pre-registration data on eleven chemicals as well as data on alternatives to 2.4.5-T

At the same time, EPA has been talking with industry representatives, who have asked for an "administrative stay" on the release of data under the Freedom of Information Act, and are said to be helping EPA to develop a set of restrictions that would be both legally defensible and commercially acceptable.

David Dickson

Moves in Mongolia

The new president of the Mongolian Academy of Sciences is Dr Choydogiyn Tseren, a 42-year-old physicist. Dr Tseren, who is a graduate of Moscow University, has spent a considerable time (1966 to 1971 and 1974 to 1980) at the International Nuclear Research Institute at Dubna. In 1980, he returned to Mongolia to become academic secretary of the academy.

Dr Tseren's appointment as president of the academy, which was effected by a special decree of the presidium of the People's Great Khural (parliament) of Mongolia, followed the dismissal, at the beginning of the year, of the previous president, Dr B. Shirendev, for his "misunderstanding" of the nature of socialism, a term which apparently covered hard currency irregularities.

Vera Rich

Coal gasification

Belgian first

Brussels

A gleam of hope for Europe's coal production came with the announcement this month that the first gasification of coal at depths of nearly 1,000 metres would take place this May in Belgium. The technique has been used for some time in the Soviet Union and in other countries but never at these depths. If the project at Thulin near the French border lives up to its promise it will turn many of the inaccessible or abandoned coal deposits in Europe into a new source of energy.

The Thulin project is run by the Institute for the Development of Subterranean Gasification, 60 per cent of whose funds are provided by the Belgian and German governments, the other 40 per cent by the EEC.

The retrocombustion technique used works by burning the coal in situ and then pushing the gas through the coal strata to a second outlet. The creation of such a circuit at this depth means working with high pressures and ensuring that the coal is permeable enough to allow the passage of the gas to the second bore hole. Air is forced down the first bore hole and ignited at a temperature of some 400°C, so that the coal ignites spontaneously.

The problem is that the deeper the coal lies the further away the second bore hole must be. At 1,000 metres, the gas would have to travel between 80 and 100 metres. The costs rise with the depth of coal deposits, as it becomes increasingly difficult to complete the circuit. Oil can be extracted at depths as low as 4,000 metres, but with coal the deeper one goes the more impermeable it is. At Thulin the problem has been overcome by forcing compressed air along the vein in order to enlarge the existing natural fissures.

The commercial applications of the technique are still uncertain, as it depends not only on energy prices but also on the coal seam thickness. Nevertheless, the British Coal Board is following the experiments with interest as a potential means of exploiting North Sea coal.

So far the gasified coal being brought to the surface in Belgium is of low quality, yielding some 2,500-3,000 kcal per m³. However, specialized treatment could increase this to 12,500 kcal per m³. At first, therefore, the gasified coal would simply be burnt in power stations but there is speculation that it could one day compete with pressurized liquid gas — now used to run cars.

The earliest date by which the technique could bring commercial benefits is put at 1990. Nonetheless the Belgian energy minister, Etienne Knoops, seizing on a spark of optimism to brighten his country's current troubles, remarked, "We are today inaugurating the birth of a new natural source of riches for our country".

Jasper Becker

Dutch energy policy

Talking shop

Waalre, The Netherlands

The steering group set up in The Netherlands to referee the national debate on the country's energy problems now finds itself grappling with financial constraints — and with some political disenchantment.

The long-running and often acrimonious Dutch energy debate began in the early seventies with the government's "energy memorandum", but got under way in earnest only last June, with the appointment of a nine-strong steering group of politicians, energy experts, scientists and labour union representatives, given the brief of organizing the public discussion.

Some years ago, a budget of £3.5 million (Df1.17 million) was allotted to the steering group to cover total costs for the two-year discussion, and the group now has a staff of 14 academics (including a theologian) and 17 administrators. Most of the money was intended for subsidies to the various groups in society seeking to work out alternative energy policies to that put forward by the government.

In January this year the steering group awarded a first round of grants, totalling £1.5 million, to 31 groups including a consortium of 50 environment groups and employers' organizations. This was only half the amount asked for by the applicants, and reflects the financial pressures being felt by the steering group. In February the government decided on a budget of around £5.5 million for the steering group instead of the £7.4 million requested, of which £1.8 million is intended for subsidies, not the £3.1 million asked for.

Discussions on the budget revealed political discontent over the policies of the steering group, discontent which at one time threatened to bring an early end to the project. Some members of parliament and press commentators were critical of the way in which the first batch of subsidies was allocated — in particular, complaining that too much money (roughly half the total) went to pro-nuclear groups having close ties with industry, and in no desperate need of the cash.

The "information phase" of the discussion is now due to be completed by the end of 1982. The aim of this part of the process is, in the words of steering-group chairman Maurits De Brauw, a former minister for science policy, "to give the public the unique chance to present alternative, scientifically based policy ideas, equivalent to those of the establishment". When that is over, a further short public discussion will follow, probably concentrating on three or four energy/economy scenarios selected from the initial phase.

Whether or not this attempt at involving all segments of Dutch society in a major

decision will actually yield some clear results remains to be seen. But parliament has at least decided to await the final report from the steering group, expected at the end of 1983, before making its final decision.

Casper Schuuring

Commercialization of research

Student dilemma

Washington

Renewed public controversy has arisen about the connections of various faculty scientists at the University of California's Davis campus with a local research company Calgene, particularly over the effect these could have on graduate students.

Last October, plant biologist Dr Ray Valentine was told by the university that he must either relinquish his position as chief investigator on a \$2.3 million grant to the university's experimental station from the Allied Chemical Corporation to support his work on nitrogen fixing (nif) genes, or give up his contracts with Calgene, of which he is a co-founder and vice-president (Nature 8 October 1981, p.417).

The university's ultimatum followed the discovery that Allied Chemical had also agreed to buy a 20 per cent equity interest in Calgene. Dr Valentine subsequently agreed to step down from the university research project. However, the publicity helped spur the state's Fair Political Practices Commission into recommending that university researchers should no longer be allowed a blanket exemption from conflict-of-interest laws routinely applied to other state employees.

The latest dispute has arisen over charges that some graduate students in Dr Valentine's laboratory have been placed in a difficult position since it became clear that some of their research on the genetics of nitrogen fixation could have significant commercial consequences.

The campus daily student newspaper last week published extracts from memoranda written by faculty members expressing concern about the impact of Dr Valentine's involvement with Calgene on the academic functioning of the university.

One set of memoranda relate to a decision by four of the five graduate students to transfer their research, with Dr Valentine's approval, to another laboratory on the campus. Dr JaRue Manning, professor of bacteriology, said that graduate students have revealed several common concerns. The first was Dr Valentine's statement that it was the students' responsibility "to meet with Calgene personnel and establish that their graduate research project is not being conducted at Calgene".

The second factor quoted by Dr Manning was what he described as the switching of student research projects as a condition for continued support after students have spent up to two and a half years on a project. "Regarding the second factor, while there is certainly a need to coordinate research activities with objectives stated in grants, etc., the manner in which these changes were made raise a number of questions as to the actions taken."

Other memoranda raise more general issues about the effects of intensifying links between university researchers and companies such as Calgene. In a note to Dr Charles E. Hess, dean of the College of Agricultural and Environmental Sciences, Dr Emanuel Epstein, professor of plant nutrition at Davis, suggests that scientists are no longer keen to exchange research data with each other as soon as it comes off the scintillation counter or the electrophoresis cell.

In another memorandum to Allen G. Marr, dean of graduate studies and research, professor of zoology Robert L. Rudd complains that plans by Calgene to initiate a research project which competes with the dissertation research objectives of one of his graduate students was "an infringement on academic freedom and potentially harmful to graduate education in general".

Both Dr Epstein and Dr Rudd said last week that they were not opposed, in principle, to collaboration between university research scientists and the private sector. As a land-grant college, the Davis campus of the University of California has long had close links with both local farmers and agricultural companies. Both, however, were concerned that public reaction to the tensions caused by the recent rush to exploit research findings in genetic engineering could lead to bureaucratic controls that might themselves be an infringement on academic freedom.

The state's Fair Political Practices Commission met on Monday to discuss whether it should give final approval to the suggestion, approved by a 3 to 2 majority at its January meeting, to require university researchers to make public any financial interest they may have in a company which is sponsoring their research (*Nature* 4 February 1982, p.357).

The internal memoranda appear to have been leaked as part of a campaign by the California Rural Legal Assistance to persuade the commission to go further and require university scientists to disclose all their financial commitments in outside companies, whether or not the companies are providing research funds to them.

Dr Valentine did not return telephone calls last week asking him to comment on the questions that had been raised about the position of the graduate students. A spokesman for Calgene said that that the company did not employ any Davis students, and was not sponsoring any research at the university. (About thirty Davis faculty members are reported to be acting as consultants to the company.)

David Dickson

Polish crisis

Questions asked

One of the casualties of martial law in Poland has been the free flow of information between Polish scientists and their Western colleagues. With the total severance of telecommunications, and the postal service considerably delayed by censorship, it has been difficult, if not impossible, for scholarly contacts to be maintained. Moreover, reports in the Western media of protest strikes in the universities and institutes of the Academy of Sciences have aroused considerable anxiety among scientists abroad as to what exactly is happening to their Polish colleagues.

The main source of information has been the lists of internees appearing from time to time via official or unofficial channels. The first list - issued by the Military Council for National Salvation was clearly erroneous; it included several names of people who were actually outside Poland when martial law was declared, such as the young chemist, Miroslaw Chojecki, who became active in the underground press after his dismissal from the Swierk nuclear research institute in 1977. Later lists, received through church channels, though fuller, are still far from complete, and evidence of a colleague's being at liberty is, by and large, negative, and deduced from the absence of his or her name on the lists to date.

Only in a few cases, like that of the 101 intellectuals who signed a letter to the Sejm (parliament) calling for an end to military rule, or the published list of academics called in for consultations with General Jaruzelski in December, is there positive evidence.

A number of scientists have been approaching Polish embassies and consulates to enquire after their colleagues. In the case of known activists, such as the mathematical logician Dr Janusz Onyszkiewicz, a presidium member of the Mazowsze (Warsaw area) branch of Solidarity, or the economist Dr Bronislaw Geremek, one of Solidarity's chief "advisers", such enquiries were initially met by suggestions that if a job could be found for them abroad, the Polish authorities would not refuse an exit visa. Following General Jaruzelski's assurance in the Sejm on 25 January that no internee would be deported from Poland, such proposals have been dropped. Nevertheless, enquiries at consulates do not proceed smoothly - officials are unwilling to discuss "rumours" — even to deny them.

On the facing page we print some extracts from such a conversation, lasting several hours, between Dr Wilfred Hodges and Dr Derrick of the British Logic Colloquium and the Polish Chargé d'Affaires Mr Gorajewski and Scientific Counsellor Mr S. Wojtaszek at the Polish embassy in London.

Vera Rich

Diplomatic conversations

What follows is an extract from a record by Dr Wilfred Hodges, of the British Logic Colloquium, and officials of the Polish Embassy in London.

Question: In his statement of 13 December 1981, General Jaruzelski said: "Our nation has enough strength and wisdom to deploy an efficient, democratic system of socialist rule. In such a system military forces will be able to stay at their proper place — in the barracks." On the other hand we have heard that some university buildings in Poland are now being used as barracks. . . Answer: Where have you heard that university buildings are being used as barracks? This is sheer rumour and we do not answer rumours.

Question: In the same statement, General Jaruzelski said: "The full list of those interned will be published." When?

Answer: Sixty-three names were published soon after 13 December, and further names have been given since on Warsaw Radio. In any case, out of the 5,906 people interned, 839 have already been released.

Question: It is reported that Solidarity members are being required to sign a "loyalty pledge" dissociating themselves from Solidarity. Can you please tell us the wording of this pledge, and what the penalty will be for those who refuse to sign? Answer: In Poland some state administrators have always been required to sign a statement of loyalty. This is nothing new, and we have heard nothing about any change in the text of the statement. As for other people being asked to sign it, and what may happen to them if they refuse, we have no sources of information apart from the British press. We have never heard mention of anybody being asked to sign a pledge which mentioned Solidarity.

Question: Will all academics who are released from internment without facing criminal charges be allowed to go back to the jobs which they held before 13 December 1981?

Answer: We don't know, because we don't know about the loyalty pledge.

Question: We have heard that the following logicians have been interned: Konrad Bieliński (Warsaw) in Biatoteka; Janusz Onyszkiewicz (Warsaw) in Strzeblinek; Marian Srebrny (Warsaw) in Biatoteka; Jan Waszkiewicz (Wrocław); Andrzej Wroński (Krakow). Can you confirm this information?

Answer: We have no list of internees. We notice that a high proportion of the people you mention were in KOR (Workers' Defence Committee). Do you have any contacts in Poland other than these people? «Answer: Yes, many.»

Question: Are the academics who are interned being allowed visits by their families? Do they have writing materials

and the use of scientific books and papers? Answer: Family visits to internees are allowed. The internment camps are resort centres, so there are reasonable facilities, but we would hardly suppose the circumstances are conducive to research. The internees get exercise and see the official newspapers. We would assume that an internee can get books and papers from his home by asking a relative to collect them.

Question: Will you allow a British logician to visit the logicians who are interned, so that he can let us know the conditions in which they are kept, and so that he can pass on to us any requests from them?

Answer: All visas issued before 13 December 1981 were automatically cancelled when martial law was imposed. The present regulations are that if anybody applies to us for a visa, he has to state which organization in Poland is supporting his visit, and then we forward the application to Warsaw for this information to be checked. A British logician could certainly apply to us, but unless he could say that, for example, the Polish Academy of Sciences was inviting him, we suppose that his request would be refused.

Question: If British logicians give you letters (either in English or in Polish) addressed to logicians interned in Poland, will you guarantee that these letters will be delivered, and that the internees' replies will be delivered to us?

Answer: Mail is allowed through now, both within Poland and between Poland and the West. But all mail between Poland and the West is subject to censorship. At a time of crisis, the bureaucrats who operate the censorship are not going to make fine distinctions; if anything doubtful comes past them, they are just going to say no. But we expect that things will get gradually better. If you want to get in touch with internees, try writing to their families.

Question: New regulations for the Polish universities have recently been announced. Are these new regulations permanent, or do they automatically end when the present emergency ends?

Answer: New regulations for the universities were under discussion before 13 December and a new bill was before parliament, but martial law prevented it from being enacted. Since it was necessary to reopen the universities somehow, we reverted to the regulations based on the 1952 law. But this is a temporary situation, and new laws on the universities will be passed as soon as possible.

Question: Which items of academic and scholarly work are to be censored? Will lectures in the universities, and letters to colleagues abroad, be censored?

Answer: Academic censorship is now the same is it was in the 1960s.

Question: After the present emergency, will scholars who are invited to visit universities or attend conferences abroad be allowed to travel outside Poland, or will permission be granted only to those who have the correct political attitude?

Answer: Even in the present emergency, some Polish scholars are leaving Poland to visit abroad. If a particular employer in Poland asks for permission for one of his employees to travel to the West, he can certainly apply, and in principle he might get permission. But visas issued before 13 December were automatically cancelled at the imposition of martial law. If there are one or two Polish scholars that you would like to have visit you, please do apply. For example, the Polish Cultural Institute in London would very likely be willing to bring over one or two Polish scholars for a meeting to be held at the institute.

Question: In 1980 the authorities in Prague cancelled a major international conference of the Association for Symbolic Logic five weeks before it was due to take place, and no clear reason has yet been given for the cancellation. Already one philosophical exchange between Oxford University and the Polish Academy of Sciences in Warsaw has been postponed by the British Academy as a result of the military rule in Poland. In this climate, are the Polish authorities aware what harm would be done to East-West scientific cooperation if the International Congress of Mathematicians, due to meet in Warsaw in August, had to be cancelled or moved as a result of the situation in Poland?

Answer: We shall try to get facts about any conferences you ask us about. In particular we shall ask whether the Polish authorities are still planning for the following conferences to go ahead: Conference in Functional Analysis, Stefan Banach Institute in Warsaw, February 1982; International Congress of Mathematicians, Warsaw, August 1982.

- •Mr Gorajewski and Mr Wojtaszek made a number of other points, including the following: (1) The Polish authorities had no option but to impose martial law, in order to protect Poland's economic links with the other countries in Eastern Europe. (2) By writing to the British Foreign Office to complain about the internal affairs of Poland, the British Logic Colloquium has violated the Helsinki Accords.
- •On receiving a copy of the above summarized answers, Mr Wojtaszek wrote: "Since many of the answers have been significantly misquoted and/or taken out of context, it would at least be fair to say that you, and you alone, might accept responsibility for the interpretations you made and which are often in sharp contrast with the contents of the answers you received."

ORRESP NIDENCE

Back to Nature

SIR — The article "Mental stress given environmental status" (Nature 21 January, p.179) shows an extreme ignorance of the dangers of ionizing radiation. Stating that there was "no significant extra radiation exposure to individuals" after the Three Mile Island incident implies the existence of insignificant levels of radiation. Not true! Any amount of radiation will produce a proportionate increase in mutation rates in exposed genes. There is no safe level of radiation, only less harmful levels.

Your leading article (Nature 21 January, p.177) regarding the recent US Court of Appeals ruling that psychological effects must be considered when making an environmental impact statement is speckled with specious statements and biased comments. First, it is ridiculous to presume that under this ruling "hydroelectric schemes of all kinds would quickly be stopped". Nuclear projects are fundamentally more dangerous than hydroelectric facilities. Dams do not increase mutation rates; dams do not remain mutagenic for thousands of years. Second, you lament that "most defence projects and all nuclear projects (would be quickly be stopped)". These structures should exist to serve the majority of the people of this country (not the armament industry or the utilities), and if the people feel traumatized by these projects to the extent of working to prevent their construction, then the people should have the power to say No. You feel that this result "would be a nonsense" and that "environmentalists (now) have a licence to do irreparable damage". I fail to see how allowing people to shape the nature of their environment can be "nonsense" or irreparably damaging to anything but the armament and utility industries. Finally, your choice of adjectives suggests an empathy with industry, not the environment. Metropolitan Edison is not "luckless". Luck has nothing to do with the scientifically and economically unsound decision to build nuclear power plants. Please, consider diverting your sympathy from the nuclear industry to your namesake: Nature.

CHRIS Q. DOE

Department of Biological Sciences, Stanford University, California, USA

Astronomical flaw

Sir — Can someone explain why creationists seem to reserve their attention for biologists and the theory of evolution and ignore astronomers?

As I understand it, the creationists reject the theory of evolution because that is not how things are described in the Book of Genesis. Yet that book clearly states that the Earth, complete with flowering plants, was created before the Sun (Genesis 1, v.9-19).

I am no astronomer but I should have thought that that was incompatible with any currently accepted theories of planet formation.

However, I have not seen the point raised in any creationist argument.

Are some parts of *Genesis* more historically true than others? If so how does one tell which is which?

I.P. FREEMAN

Beijing duck now

SIR — As reported in four articles in *Nature* of 3 December 1981¹, in 1980 foreign scientists were allowed entry into Tibet for the first time since the Chinese military takeover of that country in 1950. This opening of Tibet for scientific study is highly welcome. However, the Chinese usage of special Chinese spellings of Tibetan words (as also used in the *Nature* articles) merits comment.

Tibetan is a language quite unrelated to Chinese and has, in contrast to the latter, an alphabet. This can be very accurately transcribed using roman characters. However, Tibetan spelling being fairly complex, it is often preferred to spell Tibetan names the way they sound, so that an English-speaking reader can immediately pronounce the names with a fair degree of accuracy.

Unfortunately the Chinese now have extended the pinyin system also to Tibetan words. This system was created to romanize Chinese words in a unified manner, and it is the official system in use in the People's Republic of China today. (Thus Peking is now spelt Beijing.)

Since pinyin was constructed for the Chinese language, it can be used for Tibetan words only by a concomitant loss of accuracy. This is partly because of the system's inherent adaptation to the Chinese language, and partly because pinyin spelling is the result of a foreigner (Chinese) writing in roman letters in an artificial system what he thinks Tibetan words sound like. This filtering necessarily introduces a considerable degree of distortion.

Furthermore, pinyin replaces well-known spellings with strange ones. For instance, the second largest city of Tibet, traditionally spelt Sigatse, becomes Xigaze. Since "Shigatse" very accurately reflects the Tibetan pronunciation and is the established way of spelling the name, nothing is gained by the substitution. The same goes for changes of other words into pinyin.

Finally the Chinese system introduces a further confusion in that other Tibetan-speaking areas, such as northern India and Nepal, Bhutan, Sikkim and Ladakh, do not use this system. Thus the danger ensues that two parallel romanizations will be used for the same words.

It is to be hoped that intensified research contacts regarding Tibet can be established in the future between the West and China, and ultimately also Tibetan scientists. The question of the spelling of Tibetan words would then become of increased importance.

Jan Andersson

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1. Nature 294, 403; 405; 410; 414 (1981).

Missed the mark?

SIR — Jon Marks' letter (*Nature* 28 January, p.276) shows a misunderstanding of the intent and meaning of the Bible. The Bible is a collection of writings from various peoples and times, all focusing upon their experience of God. It is not designed to be a scientific textbook. It is wrapped within the time, culture and world outlook of its authors (what literature is not?) and yet succeeds in breaking out of those limitations to accomplish its purpose and point men to God's active concern in their lives. In this respect, Dr

Marks is just as guilty as those he criticizes in quoting the Bible in a way that is incompatible with its primary intent. He does it for ridicule; his opponents for scientific reductionism; but is either legitimate?

The way in which biblical quotations are used often tells us more about the person quoting than the subject under discussion. This is clearly evidenced by the way in which Leviticus 11:19 and Luke 23:43 were utilized. In his reference to the bat being categorized as a bird. Dr Marks has not discerned the culturally bound attempts of the writer of Leviticus to make sense out of the world based upon the evidence as he understood it. We should not stand in judgement of such attempts simply because of our more privileged information. One wonders if some of our scientific "certainties" will pass the scrutiny of scientists in four thousand years' time. Again, an understanding of the relationship between Jesus' death and the Kingdom of God in the world would prevent misinterpretation of Luke 23:43. "Paradise" as an image of the Kingdom of God is inseparably linked within the New Testament of Jesus' death and resurrection. That crucial phase of Jesus' life was beginning to take place on that very day ("today"). If the creationists and evolutionists would take time actively to study the Bible then such matters would not come to trial.

The Bible does see the design and diversity of nature as pointing to a Creator, but does not demand a fundamentalist, creationist interpretation of *Genesis* 1. Even Creationism should allow an element of evolution. Nature suggests that the Creator builds one design from another to achieve increasing complexity.

Furthermore, the Bible clearly indicates that God is still creating. The Thomistic argument of design pointing to the Creator is admittedly not without its difficulties - but equally so is the argument from chaos to non-creator. Both fall under the same criticisms and fail to see that either is dependent upon faith in establishing the relationship between data and that to which data point. Evolutionary theory has for too long been six feet above contradiction. In this context the challenges enshrined in neo-Lamarckism, punctuated equilibria theory and cladistics should be welcomed. Evolution should be subjected to the same scrutiny as any other theory, and should be capable of modification or replacement. Indeed when a theory loses its ability to be superseded it becomes a dogma.

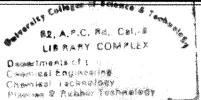
The evolutionary process, when extrapolated beyond its scientific confines, can be used to justify any number of monstrous evils, as history only too well documents. We must admit that man is a creature whose life is not encapsulated within empirical study alone but contains a drive which pushes him Godwards. Man can only be understood as making progress in so far as the touch of God is able to continue to direct him. The way forward to us seems to be commitment to scientific investigation combined with dedicated and honest study of the Bible.

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NEWS AND VIEWS



Processes of gene duplication

from Alec J. Jeffreys and Stephen Harris

GENE DUPLICATION is probably one of the most important mechanisms for generating new genes and novel biochemical functions during evolution. Evidence for this process is seen in the numerous families of related proteins specified by the genomes of higher organisms. Through the use of recombinant DNA methods to study the arrangement of duplicated genes, two basic patterns of gene family organization have emerged: linked clusters of related genes in which individual genes are separated by long regions of non-coding DNA, and dispersed families in which individual members are scattered over widely separated chromosome locations.

The globin family in mammals neatly illustrates both modes of organization, with two unlinked gene clusters, one specifying the α -related globins and the other the β -globins. The family has recently been the subject of intensive research which has attempted to discern the molecular mechanisms that lead to the tandem duplication of a single gene and to the dispersal of genes to remote locations.

The human β -globin gene cluster contains five active globin genes arranged in the order ε - ^{G}y - ^{A}y - δ - β , together with two pseudogenes and extensive tracts of DNA between the genes I . The two closely related fetal genes (^{G}y and ^{A}y) have clearly arisen by a recent duplication within the cluster. Recently, Smithies and colleagues have sequenced over 11 kilobases (kb) of DNA encompassing the fetal globin genes, and have shown that the region contains a large (5 kb) tandem duplication. Each duplicate has a γ -globin gene embedded within it $^{2-4}$.

How did this duplication of a relatively large chromsome segment arise? A clue comes from the existence of a short directly repeated DNA segment (r) at each end of the repeat, giving an organization r-Gy-r-Ay-r (ref.3). While the authors suggest several models to account for this arrangement, the simplest involves mispairing and unequal crossing-over at meiosis between the repeated elements present in an ancestral r-y-r DNA segment

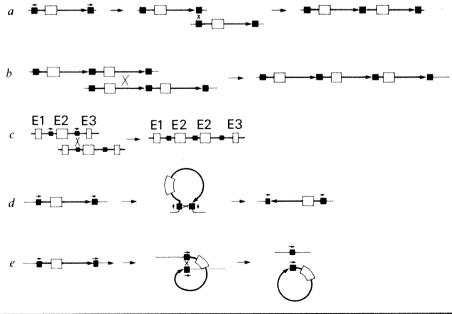
(Fig.1a). If this is correct, then the size of the duplicated region would have been initially determined by the (fortuitous?) spacing of repetitive elements near the non-duplicated locus. The prevalence of families of short repetitive elements scattered throughout the mammalian genome would suggest that large block duplications might occur commonly, though the proportion of these that would be fixed in evolution is not known.

According to the locations of the repetitive elements involved in unequal crossing-over, more than one gene could be included within the duplicated block. A fascinating example has been found by Cleary and colleagues in the β -globin gene cluster of the goat⁵. The adult $^{A}\beta$ -globin gene is preceded by a pseudogene $\psi^{Z}\beta$, and similarly the $^{C}\beta$ -globin gene coding preadult globin is linked to another closely homologous pseudogene, $\psi^{X}\beta$. The authors suggest that this arrangement has probably evolved by two successive duplications of a single ancestral β -globin gene. The first duplication, accompanied

by silencing of one duplicate, gave a $\psi\beta$ - β pair, and more recently a larger block duplication of this pair generated the $\psi^Z\beta$ - $^{A}\beta$ and $\psi^X\beta$ - $^{C}\beta$ pairs. The length of the block duplication and the presence of repetitive elements flanking the blocks have not yet been determined.

By comparing duplicated DNA segments it is possible to learn something of the modes by which genic and intergenic DNA diverge in evolution. The Gy-Ayglobin gene duplication provides an excellent example. Phylogenetic comparisons of primate β -globin gene clusters show that the duplication probably occurred about 20-40 million years ago in the lineage leading to Old World monkeys. apes and man⁶. Since then, the sequences of most of the two block repeats have diverged about 14 per cent from each other and have also accumulated numerous very small deletions and insertions2-4. Surprisingly, one substantial region (1,500 base pairs long) including most of the Gy globin gene is more than 99 per cent identical in sequence to the corresponding

Fig. 1 Consequences of recombination between dispersed repeated DNA elements. Short repeated DNA sequences (solid boxes) are shown either near genes (open boxes in a, b, d and e) or within intervening sequences separating the exons (c) of one gene. In d, the short sequences are orientated in opposite directions.



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region around the Ay-globin gene. Since it is difficult to see how selection could have led to this remarkable homology, the authors suggest that the region has been homogenized by gene conversion between misaligned y-globin repeats very recently in evolution^{2,3}. Gene conversion between related DNA sequences seems common in evolution and appears to operate not only within protein-coding gene families but also in middle and highly repetitive DNA sequences. It may serve to maintain sequence homogeneity within a family (see ref.7). It is interesting that the end of the converted region in the y-locus, as well as the end of a long homology block around the duplicated α -globin gene in man, contains the simple sequence (TG),2,8. Since this sequence can, in principle, adopt a left-handed helical configuration, the authors suggest that such an unusual localized structure in DNA might be involved in initiating the related processes of recombination and gene conversion.

In the goat, the duplicated loci each containing a $\omega \beta$ - β -globin gene pair provide an interesting test for sequence divergence of homologous pseudogenes. When homologous functional genes are compared with each other, base substitutions are found preferentially in flanking sequences, introns and third codon positions in exons, presumably because substitutions that alter the amino acid sequence of the protein are more likely to be eliminated by selection. In contrast, the goat $\psi^{X}\beta$ and $\psi^{Z}\beta$ sequences show throughout a much more uniform divergence⁵, consistent with the idea that pseudogenes are the non-functional relics of excessive gene duplication (see ref.9). However, it is worth noting that what is a pseudogene in one species can be a functional gene in another; the minor adult δ-globin gene is active in man, apes and New World monkeys but has been silenced independently in Old World monkeys¹⁰ and lemurs11.

The presence of small repetitive elements and tandem repeats in gene clusters can lead to other shifts in gene organization. Long tandem repeats provide excellent targets for unequal crossing-over, and can rapidly amplify to give arrays of three, four or many more closely related genes (Fig. 1b), such as those found in ribosomal DNA clusters, histone gene clusters and satellite DNAs. Oppositely orientated short repeats can cause localized inversions (Fig. 1d) and may have been responsible for one form of thalassaemia in man12. Dispersed repetitive elements can also exist in introns, and recombination between direct repeats in different introns (Fig. 1c) could lead to an internally duplicated intron/exon arrangement that could be further amplified by the mechanism in Fig.1b. Strong evidence for internal duplication has been found in IgC_H¹³, ovalbumin¹⁴, ovomucoid15 and a-fetoprotein¹⁶ genes, and further amplification is probably responsible for

the very highly split nature of the a-collagen gene¹⁷.

Dispersed repeated elements could also lead to excision of genes via intrachromosomal recombination (Fig. 1e). The excised circle could reintegrate elsewhere in the genome by homologous recombination with another member of the repeated DNA family. While this process might contribute to the dispersal of gene clusters without duplication, other mechanisms involving gene duplication also seem to operate. The simplest processes entail chromosome duplication, polyploidization and chromosomal translocation. A more subtle and remarkable process that might both duplicate and scatter genes is being unravelled (see A.J. Flavell Nature, News & Views 295, 370; 1982; and ref. 18). A mouse a-globin pseudogene with two remarkable properties has been found: first, it has escaped the parent gene cluster; and second, it appears to have been processed to remove introns, as though the gene had passed through an RNA splicing stage. The discovery of retrovirus-like sequences near this processed wa3-globin gene in the mouse genome suggests that the original gene was somehow kidnapped by a proretrovirus and transposed via spliced retroviral RNA.

Clearly, gene clusters are inherently unstable entities that can expand, contract and disperse during the course of evolution. Of all the possible shifts in arrangement, natural selection will eliminate the unfavourable, leaving neutral and advantageous changes to be fixed in evolution. Although it is difficult to distinguish between neutral and selective changes, some degree of adaptive changes has accompanied the Gy-Ay-globin gene duplication in man and the AB-CB duplication in the goat, in which the duplicated genes tend to be expressed at different stages of development. The molecular basis for these adaptive changes is as yet completely unknown, and is unlikely to become clear from DNA sequencing data alone.

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Parasite-induced pathology

from F.E.G. Cox

INFECTIONS with parasites are characteristically long and chronic and the resulting diseases involve diverse pathological mechanisms. The damage caused is vastly in excess of the expected local reactions to the parasites themselves and appears out of all proportion to their size. A recent colloquium* attempted to assess some of the mechanisms involved.

For many years it has been believed that the prolonged but misdirected immune responses of the host are largely responsible for the pathological changes observed. In some infections this is no doubt the case; in schistosomiasis, for example, the adult worm, coated with host antigens, is immunologically invisible but the eggs lodged in various tissues elicit classical delayed-type hypersensitivity reactions which result in the formation of granulomas, the deposition of collagen and eventually fibrosis that is often fatal. This is not, however, the whole story.

Schistosome egg antigens are themselves able to stimulate the production of collagen in a manner similar to that seen in many diseases — in particular silicosis, in which silicon compounds stimulate macrophages to produce factors that activate fibroblasts to produce collagen and eventually fibrosis. The collagen in schistosome granulomas is a normal host component produced in the liver from arginine which is used for the synthesis of proline, an essential prerequisite for collagen formation. Normally, proline is degraded but this degradation is inhibited within schistosome-induced granulomas. Collagenolytic activity is restored after the infection has been cured. Thus the major pathological lesion in schistosomiasis can be partially explained in terms of parasite by-products that influence the normal physiological activities of the host. The egg antigens also seem to be directly hepatotoxic and the granulomas actually protect the liver cells from damage, which suggests that this pathological reaction, induced by either or both egg antigens or immunological reactions to egg antigens, may have some protective effect not yet clearly understood. The clearance of foreign immune complexes is also inhibited

^{*}An international colloquium on the pathogenesis of disease induced by parasites was held at the Prince Leopold Institute of Tropical Medicine, Antwerp, on Leopoid Institute of Tropical Medicine, Aniwerp, on 11-13 December 1981. The proceedings, From Parasitic Infection to Parasitic Disease, will be published by S. Karger (Basel) in the Series Contributions to Microbiology and Immunology.

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in schistosome-infected animals; this is due not simply to changes in circulation resulting from the granuloma formation, but to the presence of adult worms. The parasites themselves are thus responsible to a large extent for the pathology associated with schistosomiasis.

In fascioliasis it is now clear that excretory products from the adult flukes have a direct deleterious effect on the liver cells of the host by blocking mitochondrial ATP synthesis. In African trypanosomiasis, the trypanosomes catabolize aromatic amino acids and not only deplete the host of essential amino acids but also produce toxic by-products from them. The main catabolites of tryptophan, for example, bring about major physiological changes including the induction of a state of coma similar to that seen in human sleeping sickness and those of phenylalanine inhibit gluconeogenesis and mitochondrial activity. In animal trypanosomiasis, extravascular parasites cause cellular infiltration and necrosis, particularly in the heart and nervous system.

Cardiac and nervous tissue are also involved in the pathology of malaria, and in rodent models, endomyocardial fibrosis following the passage of macrophages, polymorphs and fibroblasts through the endothelial layer has been demonstrated after drug cure and some brain lesions appear to be caused by the compression of capillaries by the accumulation of fluid in pericapillary glial cells. These observations indicate that physiological changes may contribute to the pathology of malaria but other evidence suggests that immune complexes characteristic of malaria simply indicate brain damage and do not necessarily cause it.

The overall conclusion is that we now know enough about the pathology of parasitic infections to say that physical and physiological changes may be as important as, or even more important than, immunologically induced damage. Of particular importance is the realization that certain aspects of the pathology of sickness resemble sleeping phenylketonuria, of schistosomiasis silicosis and of malaria, paraquat poisoning or burns. Our understanding of the pathology of parasitic infections can benefit from progress being made in other fields and should not be considered in isolation. Again, certain techniques devised for parasitological studies such as the injection of Sephadex beads loaded with schistosome egg antigens to induce liver granulomas may be relevant to the investigation of non-parasitological diseases. In this context, it is important that parasitological studies have had a leading role in our understanding of the part played by eosinophils in various infections. As eosinophils are involved endomyocardial fibrosis it may well be that the role of these cells, and the basic proteins they produce, will become more clearly

defined as a result of further investigations using parasites.

Eosinophils are not the only cells to be incriminated in the pathology of parasitic infections — macrophages and their products seem to play a central part in many infections. We need to know much more about the activities of these cells before we can say that we understand the basis of the pathology of parasitic infections let alone know enough to ameliorate the effects. Nevertheless, there is no doubt that pathology is about to emerge from years of comparative neglect when it has taken second place to immunology and will become one of the most important and rapidly developing aspects of parasitology.

A new approach to the determination of absolute configuration

from W. Jones

CHIRALITY plays an important part in chemistry and lies at the heart of many important biological processes. But under what circumstances is one kind of handedness chosen? Is a particular molecular glove intended for the right or the left hand? And even more fundamental, from which side of the mirror do we observe? These questions have been asked ever since Pasteur's classical experiment in 1848 resolved the two optical isomers of sodium ammonium tartrate¹.

Not withstanding Pasteur's breakthrough the question of absolute configuration still remained, it being necessary to make an arbitrary assignment (either right or left) to one particular molecule and refer all others to this. No further progress was made until 1949 when Bijovet proposed the use of anomalous dispersion of X rays2. His proposal rested on the fact that for an appropriate wavelength of probing X-ray beam a non-centrosymmetric crystal structure will lead to slight differences in scattering intensities from certain planes, in violation of Friedel's law. Experimental proof followed and since then the absolute configuration of over 500 optically active compounds has been determined by this method³. Apart from two other techniques³, one rather esoteric and the other somewhat restricted, but which incidentally confirm assignments based on Bijvoet's proposal, no other general methods have been available by which absolute handedness may be

W. Jones is a Fellow of Sidney Sussex College and is in the Department of Physical Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EP. determined. In this issue of *Nature* (p.21 & p.27) however, Leiserowitz and his colleages describe a new approach whereby absolute configuration can be deduced from a study of the crystal habit which a collection of the chiral molecules adopt. The approach has its origins in the reactivity of organic molecules within crystalline solids.

The course of many solid-state reactions is crucially controlled by the arrangement of the molecules within the crystal lattice4-6. Furthermore, any molecular asymmetry is manifested in the solid state by the manner in which the molecules pack in the highly ordered three-dimensional arrangement of the crystal. If both types of optical isomer are taken up within a crystal then symmetry operations which 'translate' a right-handed molecule into its left-handed counterpart (that is, a mirror plane) must be present. If such symmetry features are absent then only molecules of one handedness may be incorporated and the space group (that which describes the three-dimensional arrangement) is said to be chiral. Furthermore — and herein lies its strength - for any solid-state reaction which proceeds under so-called lattice control this chirality will be transferred to the product and only one type of optically active isomer will be formed.

By this method absolute asymmetric syntheses have been accomplished?, with the chirality of the product determined by the absolute configuration of the parent crystal. The next stage, however, is to enquire whether there exists a feedback mechanism whereby 'seeding' of the mother liquor with product of one (say D) chirality will induce preferential crystallization such that even more of the D isomer may be formed. The results indicate that the addition of D product invariably leads to growth whereby L crystals (and subsequently L product) are in excess—the inversion rule.

Leiserowitz et al. show that this occurs because the D-product isomer is preferentially absorbed on growing D-reactant crystals and impedes or considerably modifies their growth. In the absence of optically active impurities, perfect crystals of D and L form are obtained from solution. After addition of a small amount of D impurity only L crystals with perfect morphology emerge. But the full implication of these observations lies in the possibility of determining absolute configuration. For if one uses 'impurity' molecules with identified groupings and observes which crystal face or faces are modified with respect to particular directions within the crystal one has a method of obtaining absolute configuration. This argument (see p.27) leads to the emergence of a new and potentially powerful technique capable of dealing with racemic crystals and which relies only on observation of crystal morphology and very elementary X-ray diffraction measurements. The prospects are exciting.

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Not only may we find here a convenient method of ascertaining absolute configuration, but we may also reap other practical benefit from the ability, which their work presents, of engineering the morphology of crystals.

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C-reactive protein and the acute phase response

from M. B. Pepys

THE acute phase response consists of the increased production of a number of plasma proteins which occurs following most forms of tissue injury, inflammation. infection and malignant neoplasia. C-reactive protein (CRP), so-called because it precipitates pneumococcal somatic C polysaccharide, was the first 'acute phase protein' to be identified. It was discovered in O.T. Avery's laboratory at the hospital of the Rockefeller Institute and was one of three fundamental contributions to biology to emanate from there, the others being the identification of DNA as the genetic material in pneumococci and the establishment of the role of polysaccharides as antigens.

There has lately been much progress in the characterization of CRP, of other acute phase proteins and the mechanisms underlying the acute phase response in general. These advances were marked recently by the first international meeting* to be held on the subject.

Many circulating proteinase inhibitors, complement, coagulation and transport proteins have been shown to behave as acute phase reactants. The general nature of the acute phase response, its stimulation by many forms of cell injury, and its occurrence in all homoiothermic species, suggest that it fulfills a beneficial function. With respect to CRP this view is supported by the evolutionary conservation of structure and binding reactivity of the CRP

family. CRP of all species binds to phosphorylcholine (PC) residues in the presence of calcium ions and PC-binding proteins homologous with human CRP occur in many vertebrates, including fish, and even in an invertebrate, Limulus polyphemus, the horseshoe crab. A normal human plasma protein, known as serum amyloid P component (SAP) because the molecule is pentagonal in shape and because it is laid down in amyloid deposits, closely resembles CRP in structure with 60-70 per cent homology of amino acid sequence. Homologues of human SAP are also present in all vertebrate classes.

A serious pathological complication of a sustained acute phase response, due to persistent infection or inflammation, is the deposition of amyloid A (AA), an extracellular accumulation of protein fibrils consisting of peptide subunits arranged in an anti-parallel β-pleated sheet. The protein is derived from a serum precursor (SAA) which is apolipoprotein of high-density lipoprotein produced in the acute phase. The trigger molecule initiating synthesis of SAA and other acute phase proteins by liver cells is apparently interleukin-1 (IL1), a peptide product of macrophages. IL1 also activates T lymphocytes and is a pyrogen. Its production can be initiated either by direct stimulation of macrophages, for example, with bacterial lipopolysaccharide, or indirectly by mediator(s) released from damaged tissue cells, possibly prostaglandins. The mechanism by which liver cells increase the abundance of mRNA for acute phase proteins in order to provide for their increased synthesis is not yet known. However, the genes for SAA

and for α_1 -acid glycoprotein, another acute phase reactant, have now been cloned and this may provide information on control of synthesis as well as on the structure, functions and evolution of the proteins themselves.

The normal in vivo functions of CRP, SAP and SAA are not yet known. Presumably, for CRP and SAP these are related in some way to their strictly conserved ligand-binding properties. CRP binds preferentially to damaged rather than intact autologous cell membranes and having done so, human CRP activates the complement system; part of its role may be to elicit the inflammation required for resolution and repair. CRP also binds to extrinsic ligands and can thus enhance resistance, for example to pneumococcal infection in mice. In addition to being a normal plasma protein and a constituent of amyloid deposits, SAP or a closely related protein is also a normal tissue protein. It is found in glomerular basement membrane and in association with elastic fibre microfibrils throughout the body. Its role in tissues is not known but it is of interest that aggregated SAP selectively binds fibronectin from whole serum.

Clinical measurements of serum CRP. which, like basic studies of CRP itself, suffered a long period of decline, are now being treated with fresh enthusiasm. Such measurements are useful in screening for organic disease and in monitoring the activity of many inflammatory or necrosing disorders, and are a sensitive test for infection. Although the acute phase response is a non-specific manifestation of tissue injury its precise quantification can provide a valuable aid to the clinician at the bedside.

100 years ago



"Nowhere has the hand of man moulded ground into shapes more strangely contrasted with its natural form than on the site of London. Even as late as the time of Caesar the soil which a large part of it covers can have been little but a vast morass. Below Fulham the river stretched at high tide over the ground that lies on either side of its present channel from the rise of Kensington and Hyde Park to the opposite shores of Peckham and Camberwell. All Pimlico and Westminster to the north to the south all Battersca and Lamberth, all Newington and Kennington, all Bermondsey and Rotherithe, formed a vast lagoon. Near the point where the Lea poured its waters into the Thames, a traveller who was mounting the Thames from the sea saw the first dry land to which his bark could steer. The spot was in fact the extremity of a low line of rising ground which was thrown out from the heights of Hampstead that border the river valley to the north, and which passed over the sites of our Hyde Park and Holborn to thrust itself on the east into the great morass.

^{*&#}x27;C-reactive protein and the plasma protein response to tissue injury', organized by I Kushner, H Gewurz & J E. Volanskis and held at the Barbizon-Plaza Hotel, New York, 21-23 September 1981 under the auspices of the New York Acade Sciences Proceedings to be published in the Annals of the New York Academy of Sciences, 1982

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The International Conference on Venus

After Pioneer—good science, bad news

Over the last few years an intense study of the planet Venus has been undertaken following its exploration by spacecraft from the US and the USSR. At a recent meeting* in Palo Alto, every aspect of the planet was discussed. In the six articles that follow, contributors at that meeting survey a selection of the topics covered.

Our nearest planetary neighbour (its orbit has a radius seven-tenths that of the Earth), Venus has a size and mean density only slightly smaller than our own. Regrettably, even the best optical telescopes reveal a disk with an absence of surface features owing to the brightly reflecting clouds that shroud the planet.

It required high-sensitivity radar techniques to pierce the clouds and show that Venus rotates on its axis once every 243 days. Unlike all the other planets except Uranus, this rotation is in the opposite sense to the planet's orbital motion. In fact, this rotation rate ensures that approximately the same face of Venus is turned towards the Earth when the two planets are closest to each other in their orbits. This implies that during the history of the Solar System, Venus has become locked into a rotational near-resonance by the Earth's gravitational influence; Venus must necessarily be axially asymmetric in its mass distribution.

Spectroscopy revealed that the atmosphere consisted mainly of carbon dioxide and that the clouds contained sulphuric acid, while the radar measurements revealed poorly defined large-scale surface features. Several direct measurements of surface conditions were provided by Soviet spacecraft. In 1975 Venera 9 and 10 provided the only surface photographs so far obtained revealing a boulder-strewn terrain. The surface temperature was found to be over 400 °C.

Our perception of Venus underwent a revolution in 1978, however, when several spacecraft reached the planet. One was the Pioneer multiprobe mission, whose five spacecraft entered the atmosphere on 9 December and ceased to send data about two hours after entry. Four of the craft reached the surface, two on the nightside and two on the dayside. A parallel mission was the Pioneer Orbiter, which circles the planet and sends data still. In the same month that Pioneer arrived, two Soviet spacecraft, Venera 11 and 12, reached Venus, both landing a probe on the surface.

*The International Conference on the 'Venus Environment' was held in Palo Alto, California on 1-6 November 1981.

One question that was finally answered by Pioneer concerned Venus's very high surface temperature. Although closer to the Sun, the high reflectivity of the atmosphere should have resulted in a surface temperature lower than that of the Earth. Pioneer confirmed what had been suspected before - that a greenhouse effect was responsible. A carbon dioxide atmosphere would not have supplied the required blanketing effect owing to spectral 'windows' in the IR band, Pioneer showed, however, not only that enough sunlight reached the surface to fuel the effect but also that the small water (~ 0.1 per cent) and sulphur dioxide concentrations could produce enough absorption to close those spectral windows (see the article by Taylor).

Venus is sufficiently similar to the Earth and sufficiently different to provide a fascinating climatalogical laboratory. The planet's very slow rotation rate results in small Coriolis forces that might be predicted to cause less distinct latitudinal effects (such as zoning) than those found on the Earth, let alone the fast-rotating giant planet Jupiter. The absence of oceans releases fluid theoreticians from the complications of land-sea contrasts and corresponding latent heat budgets. Moreover, the planet's rotation axis is only 3° removed from the perpendicular to its orbital plane, compared with the Earth's 23°. Thus seasonal effects are virtually absent on the planet. Another difference arising from the slow rotation rate, of course, is the very slow passage of solar heating over the surface.

The picture that has emerged is discussed in detail in three of the following articles. Briefly, the temperature gradients from equator to poles are very small owing to an efficient single circulation cell in each hemisphere, which transports heat from the equator to the poles. In fact, the gradients are larger than expected. Moreover, the massive atmosphere (ninety times the mass of the Earth's), with its consequent high thermal inertia, had been expected to smooth out any longitudinal (day-night) thermal contrasts in the lower atmosphere. That this is not the case has been attributed to the action of eddy and wave transport.

The role of atmospheric waves is also thought to be crucial to understanding the high cloud velocities. The patterns are observed to super-rotate about the planet in four days, at a speed of about 100 m s⁻¹.

Above and below the three main cloud layers (which are found to concentrate at heights between 45 and 70 km) the atmosphere is relatively calm. Theorists have concluded that the high momentum is pumped into this layer by waves and eddies from the lower atmosphere, as discussed by Elson below.

The atmospheric circulation cells are concentrated at cloud heights, where much of the solar heat is absorbed. The clouds must play an important part in atmospheric energetics. It should be remembered, however, that these clouds are not the result of condensation from a vapour transported upwards by convection, as is often the case on Earth. (Pioneer found Venus's lower atmosphere to be surprisingly stable.) As Knollenberg discusses, they are the long-lived products of the atmospheric photochemistry of Venus. They have been compared with the urban 'brown clouds' which are only gradually removed from our atmosphere by rain.

Venus also differs from the Earth in that it appears to lack a magnetic field. This has important implications for the uppermost atmosphere. A strong magnetic field, as on Earth, would interact with the magnetic plasma (the solar wind) that impinges on all the planets, and deflect it. On Venus the ionized upper atmosphere on the dayside faces the wind unprotected, with the result that energy from solar disturbances can be more directly dumped from interplanetary space into the upper atmosphere. Relevant processes are discussed in more detail in the articles by Nagy and Brace and by Cloutier and Russell.

The orbiting radar of Pioneer has mapped the surface topography. These observations, combined with measurements of the planet's gravitational field, have led to the conclusion that Venus's crust is considerably thicker than the Earth's and is not broken up into 'plates' (see article by McGill). The heat from the planetary interior appears to be reaching the surface primarily beneath two elevated regions, the Beta Regio and the Aphrodite Terra highlands. The detection of volcanic land forms and radar signatures comparable with those observed from lava flows on Earth has led some to believe that these regions are currently volcanically active. This belief is supported by the discovery that the sources of lightning on Venus appear to be concentrated in these two 'volcanic' regions, suggesting that arcing

within volcanic dust clouds (as happened in the Mt St Helens eruption) may be occurring.

One of the most surprising results from the Pioneer and Venera measurements was that the abundances of non-radiogenic rare gases such as 36Ar were much higher than on the Earth. In fact, the values decrease in order-of-magnitude steps from Venus to Earth to Mars. The atmospheric abundances of these non-reactive gases result from three possible processes: (1) accretion during planetary formation from the solar nebula; (2) accretion from planetary bombardment by asteroids and comets; and (3) outgassing from planetary interiors. None of these processes would be expected to result in the observed distribution. Resolution of this problem is some way off, and is not helped by the fact

that different rare gases appear to be telling different stories.

What of the future? the next major advances in Venus science will hopefully come from the USSR. Two more Venera craft were launched at the end of last year, equipped with landers aimed at the Beta Regio area. Two further spacecraft are expected to be launched in 1984 or 1985, which will release two landers onto Venus before proceeding to Comet Halley. Meanwhile, the reduced NASA budget is impeding the further analysis of planetary data. In view of the stimulating results from Venus, such a situation can most politely be described as tragic.

Philip Campbell

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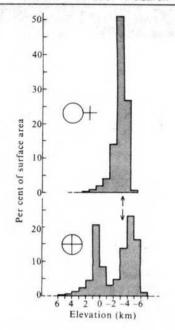


Fig. 1 Hypsographic histograms of Earth and Venus, in 1 km elevation intervals. The scale at the top is Earth elevation relative to sea level; the Venus histogram is matched to the Earth histogram at median elevations (arrows).

Geology and geophysics

from George E. McGill

Even though Venus is the most Earth-like object in the Solar System, there are important geological and geophysical differences, the most notable being the high pressure and temperature of the atmosphere (~96 bar and 740K at the median elevation)¹, and the very low abundance of water². These characteristics imply that at shallow depths, Venus is hotter and drier than Earth. Nevertheless, recent models of thermal evolution predict temperatures very similar to those on Earth in mantle regions subject to solid-state convection3-5. The high temperature of the shallow interior implies a more buoyant lithosphere than on Earth for several reasons^{6,7}, some of which depend critically on the per cent of bound H2O in the upper mantle, a quantity that strongly influences

lithosphere thickness⁸ but which remains unknown.

Pioneer Venus radar altimetry has been carried out over 93 per cent of the surface. In contrast to the bimodal distribution of surface altitude of Earth, the Venus topographical spectrum is markedly unimodal (Fig.1), with 60 per cent of the known surface within 500 m of the median radius9. The dual level of Earth topography results from (1) the contrasting densities of the two types of crustal rocks, continental and oceanic (the continental 'sial' and the denser oceanic 'sima' composed predominantly of silica and, respectively, aluminium and magnesium); (2) the Earth's plate tectonic activity, which continually creates thin, low-standing sima while sweeping thicker, higher-standing

sial into clumps; and (3) the presence of oceans that trap most sediment close to the continents. If Venus has differentiated a significant amount of sial, then the strongly unimodal elevation spectrum implies that horizontal transport of the lithosphere due to plate tectonics, if present at all, must be even slower than the presumably very slow rates of erosion and sediment transport expected on a planet with no hydrologic cycle.

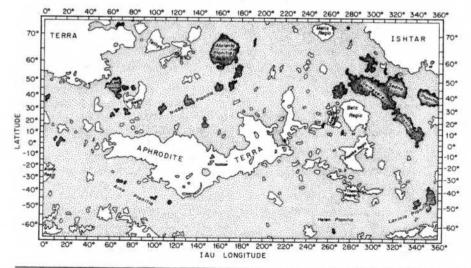
Crater-like forms occur on the venerean lowlands¹⁰; some of these may be of impact origin, some probably are volcanic, but most are enigmatic. The areally limited elevated regions include many tectonically interesting topographical features: large, straight to arcuate troughs up to 2,500 km long, round to irregular depressions, linear zones of 'pitted and hilly' terrain, and large massifs. Most of these features are associated with three regions: Aphrodite Terra, Ishtar Terra and Beta Regio (Fig. 2).

The largest elevated area is Aphrodite. In its eastern portion are several major arcuate troughs with flanking ridges⁹ and a moderately elevated terrain characterized by irregular depressions and hills (Fig. 3). The entire system of pitted and hilly terrain and troughs defines a linear zone that is radar rough at both centimetre and metre scales¹⁰ and which is almost certainly of tectonic origin¹¹⁻¹³.

Ishtar Terra includes a large plateau (Lakshmi Planum) flanked by narrow linear mountain belts along its western and northwestern margins, and by a large massif (Maxwell Montes) along its eastern

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Fig. 2 Major topographical regions of Venus. Elevations >1 km above median clear; elevations within 1 km of median in light stipple; elevations >1 km below median in heavy stipple. Mercator projection greatly exaggerates the size of Ishtar Terra, which actually includes about half as much area as Aphrodite Terra.



margin which contains a point 11 km above median — the highest so far discovered on Venus. On the flanks of this massif are several depressions, including a circular region smoother at centimetre scale than its surroundings14. The depressions are probably volcanic in origin¹⁰. Two larger but much more irregular depressions occur on Lakshmi Planum. Their obvious noncircularity and their association with large 'canyons' extending into the plateau from its southern rim constitute strong evidence that they are of tectonic or volcanic origin rather than due to meteorite impact 12,13. The general association of a large plateau. volcanic and tectonic depressions, and linear mountain belts has led some workers to suggest that Ishtar is the best candidate for a true continent on Venus^{4,5}.

Beta Regio appears to be a domical uplift with a medial rift, and with at least one large shield volcano built on it14,15. This association, together with some additional topographical details, lead naturally to the inference that the Beta Regio area is analogous to a continental rift system, such as in East Africa 12,13,16.

Despite the presence of some landforms suggesting active tectonics, there is no evidence of a global system of spreading ridges and subduction trenches characteristic of Earth's plate tectonics even though the resolution of the Pioneer Venus altimetry was adequate to resolve them had they been present 17. If the tectonic features described above are assumed to be part of a smaller-scale plate tectonics system, it can be shown that such a system would be grossly inefficient in removing heat from the interior of Venus4,5,18.

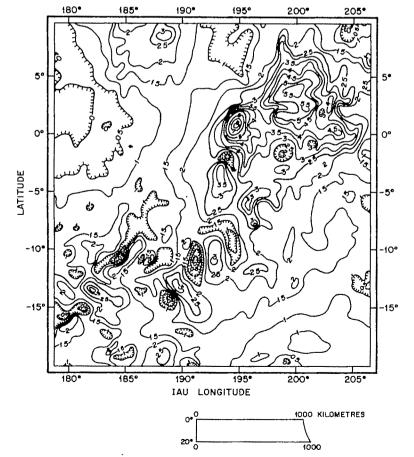
Surface properties and processes also provide some limited data bearing on the geological evolution of Venus. Igneous rocks on Venus are expected to contain minerals that are essentially identical with those found in common rocks on Earth (possibly excepting hydrous phases)12,13. These minerals should be chemically weathered by the venerean atmosphere. particularly at high elevations¹⁹. Aeolian transport of weathering products can result in atmosphere/rock chemical cycles because of the large variations in atmospheric pressure and temperature (\sim 60 bar and \sim 110K) that accompany the >13 km relief on Venus^{1,9}. Expected threshold friction speeds appear fully sufficient to erode and transport particulates given the $\sim 1~\text{m s}^{-1}$ free-stream wind velocities measured by Venera and Pioneer probes near the surface 12,13,20-22.

Earth-based and Pioneer radar determinations of the Fresnel reflection coefficient indicate that the surface of Venus cannot be covered by a thick (more than a few centimetres), areally extensive layer of porous regolith or aeolian sediment^{12,13}. It is possible, however, that

aeolian sediment is cemented by chemical reaction with the atmosphere, a process that would increase its bulk density and render it indistinguishable (to radar) from slightly weathered igneous rocks. Very high reflectivities that imply densities consistent with fresh mafic or ultramafic igneous rocks occur in only a few areas of high elevation, especially the southern part of Beta Regio²³. Presumably these areas are very young, or they are sites of active erosion.

Although both topographical data and thermal modelling predict that an Earthlike system of plate tectonics is not present on Venus now, the abundance of 40Ar in the atmosphere of Venus² suggests that the overall rate of tectonic and volcanic activity during its evolution has been only a little below that of Earth. Moreover, longwavelength gravity anomalies coincide in position with major topographical features, a correlation that, considering the hot lithosphere, suggests dynamic mantle support of the topography^{4,5,18}. How then is heat lost from the interior of Venus? Phillips (Lunar and Planetary Institute) suggests a tectonic style similar to intraplate, hot-spot tectonics on Earth^{4,5}. Perhaps Venus is characterized by a global system of domes, local rifts and active volcanic centres, with specific elements developing and decaying in concert with changes in the mantle flow patterns responsible for them, implying that little or none of the modern topography of Venus has survived from primordial times. This model suggests that modern Venus may well be tectonically similar to Archaean Earth.

Fig.3 'Pitted and hilly' terrain in eastern Aphrodite Terra. This terrain is much rougher to radar at both centimetre and metre scales than are the lower regions in the north-west and south-east corners of the map. Contour interval 0.5 km; datum is median radius.



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Structure and energetics of the atmosphere

from F.W. Taylor

For many years now, the key question about the structure of the atmosphere of Venus, and the mechanisms that shape it. has been the origin of the high surface temperatures and pressures (over 750K and nearly 100 bar respectively). It is now reasonably certain that the atmospheric 'greenhouse effect', whereby incoming short-wavelength solar radiation penetrates the clouds more efficiently than outgoing long-wavelength thermal radiation, traps enough heat to account for the severe conditions in the lower atmosphere. The wealth of new data from the recent Pioneer Venus and Venera missions has placed calculations of the greenhouse model on a much firmer footing than previously, with measurements of the amount of solar heating at different altitudes, the vertical distribution of cloud opacity and the total IR flux escaping from the top of the atmosphere.

About 2.5 per cent of the energy from the Sun intersected by Venus reaches all the way to the surface. About 80 per cent is reflected back into space by the very reflective sulphuric acid clouds without being absorbed by the planet at all. Of the remainder, about half is absorbed above 60 km altitude (mostly near the very top of the clouds) and the rest below. It is somewhat disturbing that the best calculations of the thermal component of the 'greenhouse' still allow about 200 W m-2 to escape from the top of the atmosphere on average, whereas only 158 W m⁻² was observed by orbiting IR sensors. This suggests that there is a fairly important, but still unidentified, source of opacity in the clouds. However, the relatively low actual flux out makes it easier to 'balance' the greenhouse calculations at the required high surface temperatures. A further unresolved problem is that albedo (or reflectivity) measurements from the Earth and from space indicate that Venus keeps, on average, only about 145 W m⁻² of the flux from the Sun, raising questions about where the other 10 per cent or so comes from. The albedo is probably less than the present best value of 0.80 indicated by a preliminary analysis of the data, and it is being re-examined.

The most spectacular new features emerging from the recent rash of exploration lie in the atmosphere at the cloud tops and above. At these levels the radiative and dynamical time constants are less than the venusian day and interesting diurnal (daynight) and seasonal (equator-pole) structure has emerged, much of it poorly understood. At the highest levels, in the part of the atmosphere called the exosphere (Fig. 1), the temperature varies from 300K

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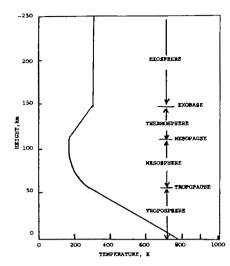


Fig.1 Nomenclature for the different atmospheric regions on Venus, based on the mean temperature structure by analogy with Earth. The thermosphere and exosphere are also known collectively as the 'upper' atmosphere, the mesosphere and troposphere as the 'middle' and 'lower' atmosphere respectively.

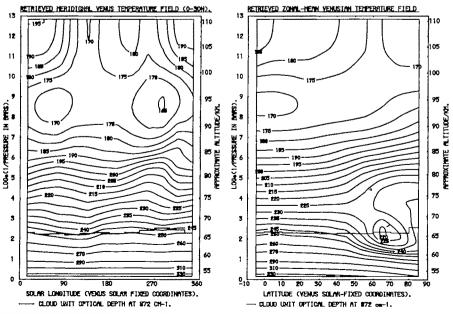
on the dayside (cooler than predicted by models) to a surprisingly low 100K on the nightside. It is very difficult to find cooling mechanisms efficient enough at these low pressures to explain the latter at all, and the very sharp drop which is observed across the terminator enhances the problem. At lower levels near the mesopause (Fig. 1), the temperature distribution is more nearly as expected, with 'warm' air (185K) near local noon, a 'cool' (170K) nightside and an

'intermediate' (175K) pole. However, the simple pattern is upset by a 'hot spot' of 185K near local midnight, where solar heating is a minimum. This is thought to be produced dynamically, perhaps by compressional heating in the descending branch of a symmetric sub-solar to antisolar circulation pattern. A little below the mesopause level, the distribution of temperature alters radically. At levels near 90 km altitude, the sub-solar point is the coolest on the planet and the poles are warmest. This remarkable state of affairs remains largely unexplained, although theories abound. These include absorption of solar radiation in a 'polar cap' of mesospheric haze, a 'hotplate' effect due to depression at the poles of the main cloud to warmer levels, and again dynamical heating, this time in the descending branch of a hemispherical Hadley-type cell. The reversed equator to pole temperature gradient persists nearly down to the cloud tops (Fig.2).

An interesting feature of any atmosphere is its response to the diurnal cycle of solar heating -- the thermal tides. Instead of the familiar wavenumber one fluctuation of the Earth's atmosphere (single maximum and minimum temperatures around an equatorial circumference), Venus surprises with a wavenumber two tide. Near the cloud tops, temperatures peak some 5K above the mean value in the morning and again in the second half of the night, with minima in the afternoon and post-duck quadrants. Why does Venus respond to predominantly wavenumber one solar forcing with a wavenumber two tide? Theorists are beginning to develop tidal models which are outlined below by Elson.

Even more obscure are the mechanisms which give rise to the two most pro-

Fig.2 (left) An equatorial cross-section through the mean atmospheric temperature field on Venus, from Pioneer Venus IR soundings. 0° solar longitude is the local noon, 90° the morning terminator and so on (remembering that Venus rotates in the retrograde sense). The stepped line near the bottom of the figure is the height of the main cloud top as a function of local time on Venus. Note the thermal tides. Fig.3 (right) As Fig.2, but an equator to pole cross-section (0° = equator, 90° = N pole). Note the polar warming and the circumpolar collar.



minent phenomena of Venus's atmosphere, the polar dipole and the circumpolar collar. The true nature of these was hidden from Earth-based observers for decades because they occur at such high latitudes (65–70° and 80–90° respectively) as to be foreshortened beyond recognition to all but the polar-orbiting Pioneer Venus Orbiter. They appear in PVO IR images as a very cold, nearly circular feature about 5,000 km in diameter centred on the pole (the collar) and two hot features, thought to be holes in the otherwise ubiquitous cloud cover, about 1,000 km either side of the pole and rotating around it (the dipole; see Nature 279, 613; 1979). A meridional cross-section of the temperature structure of the middle and upper atmospheres (Fig.3) reveals the collar as an intense, cold planetary-scale wave of long duration. It has been speculated that it is a disturbance produced at the boundary between the 'solid body' rotation of the atmosphere, which is observed in cloud-tracked winds and latitudes equatorwards of the collar, and the regime polewards in which the atmosphere accelerates in the zonal direction as it flows towards the pole, in an attempt to conserve angular momentum. This acceleration is evident in the high rotation rate (every 2.5 to 3 days) of the dipole at the centre of the collar. The dipole is the eve at the centre of the polar vortex on Venus, remarkable for its 'double' appearance. Two clearings in the polar cloud orbit around the pole, rather than the expected one at its centre. The dipole itself is perhaps the biggest mystery of Venus' atmosphere. and a prime target for future missions to

may reappear above 100 km and, as was discussed by Mayr, it seems to be required at altitudes above 150 km to explain Pioneer observations.

Atmospheric thermal tides, temperature and wind perturbations caused by solar heating of the atmosphere were addressed by several speakers. Elson discussed them from an observational and diagnostic point of view, suggesting that the temperatures observed by the Pioneer spacecraft are difficult to reconcile with our terrestrial experience, and that model calculations indicate that the tidal winds are probably small in amplitude and dominated by dissipative forces. Pechmann (California Institute of Technology) described theoretical calculations of tidal winds and temperatures using a model. Her results suggest that the tidal response of the atmosphere strongly depends on the mean (zonal and time averaged) zonal wind and static stability. A similar calculation of the tidal fields was made by Fels (Princeton University) but for a very different purpose. Assuming that the unusual observed warming at the poles has a nonradiative source, Fels chose to examine a dynamical forcing mechanism which relies on the interaction between tidally forced waves and the averaged flow whereby the tides force the averaged flow to descend in the polar regions. This descent produces warming at the pole due to adiabatic compression of the atmospheric gas as it moves down to higher pressures. To produce warmings as large as are observed, however, this mechanism must assume that the cloud level winds are much stronger than those observed.

Tides were not the only type of waves to be discussed during the session. Apt (Jet Propulsion Laboratory) reported the presence of waves with periods of 5.3 and 2.9 Earth days over the entire Northern Hemisphere between the cloud tops at about 65 km and 90 km. These waves are thermal perturbations observed at IR wavelengths. Del Genio (NASA Goddard Institute for Space Studies) reported the existence of a 5.2 day wave from UV observations. Perhaps there is a temperaturesensitive UV absorber present. In the area of theoretical wave calculations, a presentation was made concerning results produced by a general circulation model of the type used for comprehensive terrestrial studies. Young (NASA Ames Research Center) described calculations of baroclinically unstable waves whose terrestrial counterparts are responsible for most of what is called weather. As is the case on the Earth, these waves are caused in part by the vertical shear in the zonal wind. Despite this similarity, differences in the rotation rates and atmospheric structures of the two planets appear to produce different wave characteristics.

Dynamics of the atmosphere

from Lee S. Elson

ATTEMPTS to explain the observation that the entire atmosphere of Venus moves in the same retrograde (westward) direction provided one important focus of the conference. Schubert (University of California, Los Angeles, and see refs 1, 2) described the atmospheric circulation as it is now known from the Pioneer Venus and the Soviet Venera spacecraft. The atmosphere moves westwards at average speeds of up to about 110 m s⁻¹ and there is a meridional (north/south) cloud level cell analagous to the terrestrial Hadley cell. As shown in Fig.1 (from Schubert's paper). there is considerable variation in this flow with time. Therefore, the term '4 day wind' used to describe the periodicity of UV features advected by the retrograde winds is a misnomer, because the wind speed corresponds more closely to a 5 day period.

Several suggestions for the possible causal mechanisms of this flow were made in the context of comparative planetary meteorology. Mayr (Goddard Space Flight Center) and Williams (Princeton University) considered the role of meridional cells, viscosity and solar heating of the atmosphere in producing zonal (east/west) jets on Jupiter, Saturn and Venus. Their conclusions and the discussion with the audience indicated that one might expect such jets on Venus if several mechanisms operated simultaneously. However, model calculations are still unable to reproduce exactly the observed flow while simultaneously satisfying heat and momentum conservation constraints. Two papers dealing with the zonal jet above the clouds were also presented. Elson (Jet Propulsion

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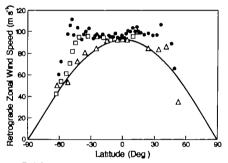


Fig. 1 Mean cloudtop westward wind as a function of latitude. Mariner 10 data from 1974 (filled dots) show different structure from that of Pioneer Venus as shown by the open symbols representing UV data presented by Del Genio.

Laboratory) described evidence³ that the zonal jet dies off with increasing altitude so that it has disappeared entirely above about 80 km altitude (see Fig.2). The jet

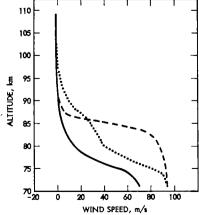


Fig. 2 Mean westward wind as a function of altitude above the cloud tops. The solid curve is the best estimate based on a model 4 and the other curves are the results of a study of the model sensitivity to input assumptions.

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Clouds and hazes

from R.G. Knollenberg

THE Venus cloud and haze system summarized in Table 1 is planetary in nature with an enormous vertical extent of 50-60 km. The clouds are rather tenuous and were found to have an average visibility of about 1 km by early Venera nephelometer (particle backscatter photometer) measurements1. The main cloud deck is at an altitude of 45-70 km and includes particles of various compositions². Upper, middle and lower cloud regions are easily defined by transition layers and changes in size properties. Haze layers overlie and underlie the main cloud deck.

Through an analysis of polarimetry data, Kawabata has shown that the upper submicron aerosol haze has an average optical depth of about 1 in its most dense polar regions where it is a distinct layer above the main cloud deck3. In the equatorial and mid-latitude regions, it is about one-tenth as dense and is mixed with 2 μ m diameter monodispersed H2SO, cloud droplets at the top of the main cloud deck. The haze particles also have optical and physiochemical properties consistent with H,SO.

Ragent and Blamont's nephelometer data show a sharp base for the main cloud deck at 47-50 km at four different Pioneer Venus entry sites but thin filaments are also evident below the nominal cloud base4. Knollenberg and Hunten have shown that the size distribution is generally multimodal throughout the main cloud deck, which has an optical depth of 20-30 (ref.5). There are three size modes identified as mode 1 aerosol, which varies in composition with altitude; mode 2 H2SO4 droplets; and mode 3, which appears to include H2SO, droplets and perhaps crystals of unknown composition. Below the main cloud deck, small haze particles of low number density extend down to approximately 30 km, where all particles

Mode 1 is highly variable in all cloud regions and has been characterized as a background aerosol population with high number density but little optical depth except in the UV. Toon has shown that, in the upper cloud region, the mode 1 aerosols have a higher refractive index and seem to be rich in sulphur compounds⁶. In the lower cloud region, H2SO4 appears more plausible. Below the cloud deck, the mode 1 aerosols, which comprise the lower thin haze, appear to be desiccation products (cores) of cloud particle growth and decay.

The mode 2 H₂SO₄ droplets are probably several months old in the upper cloud region unless vertical exchange is faster than suspected, but perhaps only

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Table 1 Summary of Venus cloud and haze properties

Region	Altıtude (km)	Temperature (K)	Optical depth T	Av. no. density (per cm ³)	Mean diameter (µm)	Composition
Upper haze	70- 9 0	225-190	0.2-1.0	500	0.4	H ₂ SO ₄
Upper cloud	56.5–70	286–225	6.0-8.0	(1)-1,500 (2)-50	Bimodal 0.4, 2.0	H ₂ SO ₄ + sulphur
Middle cloud	50.5-56.5	345–286	8.0–10	(1)-300 (2)-50 (3)-10	Trimodal 0.3, 2.5, 7.0	H ₂ SO ₄ + crystals (?)
Lower cloud	47.5–50.5	367-345	6.0–12	(1)-1,200 (2)-50 (3)-50	Trimodal 0.4, 2.0, 8.0	H ₂ SO ₄ + crystals (?)
Lower baze	31-47.5	482–367	0.1-0.2	2-20	0.2	H ₂ SO ₄ + acrosol
Pre-cloud layers	46, 47.5	378, 367	0.05, 0.1	50, 150	Bimodal 0.3, 2.0	H ₂ SO ₄ + aerosol

hours old in the lower cloud region. At upper altitudes, H2SO4 droplets are likely to be contaminated after scavenging aerosol over their long lifetime. Near cloud top, H₂SO₄ vapour should be sufficiently supersaturated to support a bimodal population; that is, small droplets can nucleate and grow even in the presence of an existing larger droplet population. The mode 2 H2SO4 droplets nucleate and grow in the upper cloud region. Knollenberg and Hunten observe a gradual increase in number density descending through the main cloud deck. The mode regenerates through condensation in the lower layers. Its size distribution is exceedingly narrow at any one altitude — growth models of Turco and Toon indicate that dominant but competitive droplet diffusional growth can explain this⁷. The mode 2 distribution is uniform over most of the planet and is indicative of its extreme stability.

Analysis of particle image data by Knollenberg and Hunten showed the largest mode 3 particles to be asymmetric and suggested a crystalline species interpretation. Toon has shown that the radiative flux and single particle optical data are consistent without a crystalline species⁸ and favours H₂SO₄ droplets. Additional measurements will probably be required to resolve this controversy.

The entire Venus cloud system has an optical depth of 20-35 at visible wavelengths. Taylor reports a radiometric albedo of 0.77-0.82, increasing from equator to pole9. His IR radiometer data have revealed a strong dipole circulation near the poles. Tomasko finds single scattering albedoes ranging from a low of 0.995 in the upper cloud region to 0.999 in the lower cloud region¹⁰. For most of the radiometric spectrum the planet appears featureless; however, fascinating contrasts are observed in the UV via partially understood absorption processes. Pollack and Esposito find that sulphur dioxide is responsible for absorption at short UV wavelengths, but the identity of the absorber visible from Earth in the near-UV

remains unknown^{11,12}. Particulate sulphur (mode 1) appears to be a possible choice for secondary absorber. The related question of how the physical and/or chemical processes at the cloud top act to bring about the horizontal variability of these absorbing constituents is still a puzzle.

The main cloud deck is embedded in the zonal circulation at altitudes of greatest wind velocity and vertical wind shear. The clouds are all of stratiform morphology. Cloud particle growth is not strongly influenced by either the large-scale circulation or the latent heat released during condensation. The clouds are most strongly influenced by radiative exchanges. Because of the temperature extremes, there is a rather large scale of H₂SO₄ droplet growth times ranging from months in the upper hazes to hours in the lower cloud region. There is little evidence for the existence of large precipitation particles. Known processes for the formation of the lightning observed by Ksanformaliti and Scarf^{13,14} require both. If cloud processes generate the lightning, then much larger undetected particles must exist. Scarf has suggested that the Venus lightning is phaselocked with the surface and may be solely the result of volcanic activity 15.

essentially disappear.

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Structure and dynamics of the ionosphere

from A.F. Nagy and L.H. Brace

A general view of the ionospheric structure of Venus and of the major processes operating there are shown in the figure. Solar extreme ultraviolet (EUV) radiation falling on the thermosphere creates the daytime ionosphere, which is in photochemical equilibrium near its peak at about 142 km, where the major ion is O2+; transport processes become dominant above about 200 km, where the major ion is O⁺. The upper ionosphere is in near diffusive equilibrium much of the time, although ion transport to the nightside and the loss of plasma through the ionopause frequently cause departures from diffusive equilibrium at high altitudes and in the region near the terminator. A comparison of calculated and measured ion composition indicates that we have achieved a first-order understanding of the very complex photochemical processes involved. Recent suggestions for the inclusion of a large number of specific reactions involving metastable species have even further narrowed the differences between the models and the experimental results. The dayside ionosphere is heated internally by solar EUV-generated photoelectrons and heated from above by the downward conduction of solar wind energy deposited at the ionopause. The ion gas also receives heat from exothermic chemical reactions

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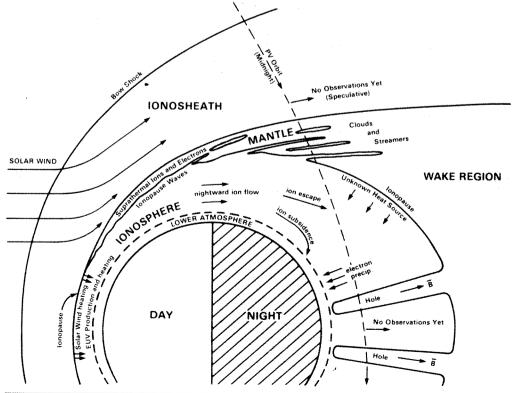
The day-to-night plasma pressure gradient across the terminator drives a nightward flow of ions, usually at supersonic velocities; some of these ions subside to contribute to the formation of the nightside ionosphere. The great variability of the nightside ionosphere is believed to arise from changes in the proportion of the flow which is lost to the wake. Even without the nightward ion flow, a nightside ionosphere would be formed by the precipitation of electrons with energies up to a few hundred electron volts, although the measured electron fluxes do not appear adequate to be the only source of the observed nightside ionization.

Large-scale radial holes or plasma depletions extend downwards to nearly the peak of ionization in the antisolar region. These holes, which occur in north-south pairs, enclose regions of strong radial magnetic fields that are believed to originate in the magnetotail of Venus. The holes or, more precisely, the radial magnetic fields act as a barrier to the flow of ionization into the antisolar region and may be related to the greatly elevated ion temperatures observed there. It has been suggested that the holes are formed by the removal of plasma by outward acceleration caused by magnetotail-generated electric fields.

The ionopause itself is a highly dynamic and complex surface which extends from an average altitude of 290 km at the subsolar point to about 1,000 km at the terminator. Its altitude varies from 200 to

more than 3,000 km on the nightside. though its height in the antisolar region will not be measured until further increases occur in the Venus orbiter periapsis altitude during the next few years. The increase in ionopause altitude from the subsolar point towards the terminator reflects the decrease of solar wind dynamic pressure with solar zenith angle. The ionopause altitude is highly variable, with a time constant of probably less than one hour. On the dayside, a near static balance between the solar wind and ionospheric plasma pressures is maintained, which results in an ionopause altitude variation which is inversely proportional to the wind pressure. The solar wind pressure is conveyed to the dayside ionosphere primarily by means of an enhanced magnetic field approximately parallel to the ionopause surface, although some of the solar wind plasma may interact directly with the ionospheric plasma.

Just above the ionopause is the mantle, a transition region between the ionospheric plasma and the flowing shocked solar wind plasma. A variety of solar wind interaction products are observed in the mantle, including suprathermal ions and electrons, ionospheric photoelectrons, enhanced plasma wave activity, and clouds or streamers of ionospheric plasma that appear to be swept downstream by ionosheath flow. These features are probably signatures of the plasma processes that form the ionopause and divert the solar wind about Venus; however the specific processes have not yet been identified.



Current views of the ionospheric structure of Venus, showing the major processes that are believed to be operating in the region. The scale of the ionosphere is increased by a factor of three relative to the ionosheath for clarity of presentation (reproduced from Brace, L.H. et al. The Ionosphere of Venus: Observations and their Interpretation, Venus, University of Arizona Press; 1982).

The solar wind interaction

from P.A. Cloutier and C.T. Russell

VENUS provides our only well documented example of the interaction of supersonic or, more correctly, supermagnetosonic magnetized plasma with an unmagnetized and highly-electrically conducting ionosphere. The Pioneer Venus observations confirm the zero-order picture derived from the earlier Venera and Mariner missions as sketched in Fig.1. A bow shock forms in front of the planet which heats, slows and deflects the flow around the ionosphere. The magnetic field is draped over the obstacle and increases in strength towards the planet while at the same time slipping around the planet as the plasma flows out of the plane of the figure. The physics by which this behaviour takes place is fairly well understood. The interesting physics, and that unique to Venus, at least so far, involves perturbations of this picture. These perturbations provided much of the controversy in the discussions of the solar wind interaction on the last day of the Venus conference.

Venus has a magnetic tail very like a comet. The Pioneer Venus magnetometer shows that behind Venus, in a region about 4 Venus radii across, the magnetic field is parallel to the solar wind flow and enhanced above interplanetary levels. Barnes (NASA Ames Research Center) and Intriligator (Carmel Research Center) reported that in the distant tail, interesting plasma effects are seen, including regions of depletion of solar wind plasma and regions of pick-up of ionospheric oxygen ions. Closer to the planet, Barnes observed the acceleration region for the oxygen pickup. Near the terminator, the oxygen flow was weakest and fastest at high altitudes, growing in density and decreasing in speed as the planet was approached.

The source of this oxygen is presumably charge exchange and photoionization of the hot neutral oxygen exosphere at altitudes above the normal ionosphere. At high altitudes, this new cold plasma is accelerated either by the solar wind electric field or by magnetic stresses and escapes from the planet. At low altitudes, in the ionosphere, the magnetic and electric fields are weak and new ions remain bound by the planet's gravitational field. It is difficult to assess how important these processes are. Vaisberg and Zeleny (Space Research Institute. Moscow) have modelled mass loading of field lines due to photoionization in a cylindrical geometry but find that the flux of oxygen is too low. Perhaps charge exchange will provide the

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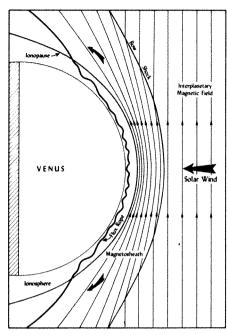
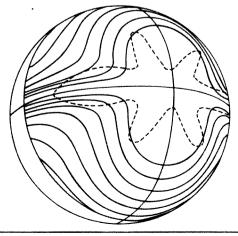


Fig. 1 Sketch of the interaction of the magnetized solar wind plasma with Venus's unmagnetized and highly-electrically conducting ionosphere.

missing flux but they prefer a source of anomalous ionization. Another indicator of the importance of these non-magneto-hydrodynamic processes is the position (Slavin, UCLA, and Mihalov, NASA Ames Research Center) of bowshock. The shock is somewhat weaker and closer to Venus than would be expected if all the solar wind were deflected by the planet. Charge exchange could provide the requisite removal of momentum from the flow.

Fig. 2 Magnetic field line configuration on Venus's dayside (see the text). The dashed line shows the boundary separating regions stable and unstable to the Kelvin-Helmholtz instability.



Some of these phenomena were anticipated by a minority of scientists before the latest barrage of data from Pioneer Venus. Many, however, were not. Two unexpected phenomena were discussed by Brace (NASA Goddard Space Flight Center): plasma clouds, dense regions of cold plasma apparently detached from the ionosphere proper in the terminator region and ionospheric holes, regions of density depletion accompanied by radial magnetic field in the near midnight ionosphere. Another unexpected feature of the dayside ionosphere is the existence of magnetic flux ropes, twisted bundles of magnetic flux which often pervade the dayside ionosphere. An artist's conception of the occurrence of such a rope is given in Fig.1.

Flux ropes and other observed features of the dayside ionospheric configuration may be explained as resulting from induced convection within the ionosphere driven by the solar wind interaction. In a model proposed by Cloutier (Rice University) and his colleagues, pressure gradients and electric fields induced in the ionosphere drive an anti-sunward convection pattern which carries ionization and the draped interplanetary magnetic field towards the anti-solar point. In the model, velocity shear within the ionospheric convection pattern leads to flux rope formation due to the onset of the Kelvin-Helmholtz instability. Calculations of the ion convection patterns, the field geometry and the extent of the Kelvin-Helmholtz unstable regions reproduce many of the observed characteristics of the dayside Venus magnetic field behaviour, including the presence or absence of flux ropes, the occurrence of large magnetic fields at low altitudes, and the lack of coincidence of times between the ionopause and large magnetic gradients. The field line configuration is shown in Fig.2 by the solid lines, and the dashed contour shows the boundary between region stable and unstable to the Kelvin-Helmholtz instability. Flux rope formation may occur outside of the dashed contour.

The importance of the Kelvin-Helmholtz waves at the ionopause was discussed by Curtis (NASA Goddard Space Flight Center) who described the acceleration of ionosheath plasma by parallel electric fields arising from conversion of Kelvin-Helmholtz wave energy into a kinetic Alfvén wave. Curtis also showed that ions and electrons may be thermalized to high energies by high levels of plasma wave turbulence at Venus.

Finally, there are still some classical interaction problems to be solved: how, for example, does the flow close behind Venus (Knudsen, Lockheed), and is the interaction viscous rather than inviscid as preferred by Knudsen (Pérez-de-Tejada, Institute Geofisica, Mexico). In all there was a great deal of controversy and evidence of many more important principles to be uncovered in the solar wind-Venus interaction problem.

ARTICLES

Resolution of conglomerates by stereoselective habit modifications

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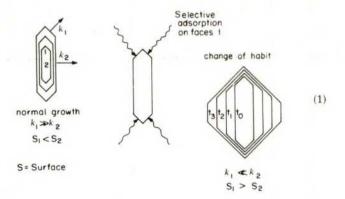
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A method for resolution of conglomerates, based on morphological differences between the enantiomorphic crystals induced by tailor-made resolved impurities is described. The relationship between the crystal structure of the affected enantiomorph and the stereochemistry of the added impurity is analysed in the systems glutamic acid. HCl, asparagine. H_2O and threonine in the presence of L- or D-amino acids, and it is found that the additive binds stereoselectively at the surface of the enantiomer of the same absolute configuration, occupying the same site of the amino acid groups of the host molecule. The modified side chains perturb in a second stage the regular growth of the crystal in the directions along which the side chains of the host are hydrogen bonded.

IN 1848 Louis Pasteur found that racemic sodium ammonium tartrate crystallizes as two monohydrates of enantiomorphous habits¹. His separation of these two types of crystals and the observation that in solution they showed optical rotations identical to those of solutions of the separate enantiomers established the foundations of modern stereochemistry.

This crystallization behaviour might have escaped Pasteur's attention had not the enantiomeric crystals in this system been readily distinguishable visually: the 'laevo' and 'dextro' crystals have particular 'hemihedral' faces, which make the habits enantiomorphous². This experiment is remarkable because the combination of such 'spontaneous resolution' with visual distinguishability ('hemihedrism') is rare³, with less than a dozen examples having been recorded⁴.

Our recent discovery of a new and general method for kinetic resolution of conglomerates also allows the assignment of absolute configuration of chiral molecules^{5,6}. In connection with a study on generation and amplification of optical activity in prebiotic conditions^{7–9}, we found that the crystallization of a conglomerate $\{R\}+\{S\}$ is influenced in a dramatic way by the presence of small amounts of a resolved impurity S'. Selective adsorption of the additive S' on the growing $\{S\}$ crystals, of similar stereochemistry, induces a substantial decrease in growth rate of these crystals, allowing their kinetic separation from the unaffected $\{R\}$ crystals. This same effect causes the $\{S\}$ crystals to undergo, during growth, morphological changes which can be extreme ^{10–14} and which allow easy visual identification and separation of the two enantiomorphs scheme (1).



This phenomenon offers the possibility of modifying the classical Pasteur experiment and of extending it to systems which undergo spontaneous resolution but do not display hemihedrism³.

We describe here the morphological aspects of this selective adsorption process and discuss how they affect the action

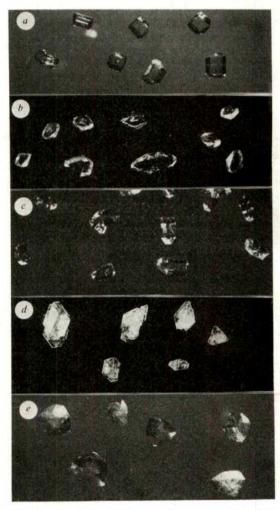


Fig. 1 Crystals of Asn. H_2O grown in presence of additives: a, none, or D-Asn + (L) additive; b, L-Asn + L-Gln; c, L-Asn + L-Ser; d, L-Asn + L-Asp; e, L-Asn + L-Orn. In each case the crystals were obtained from D, L-Asn as a mixture of D-Asn displaying a morphology as in a, and L-Asn as in b, c, d or e, depending on the impurity.

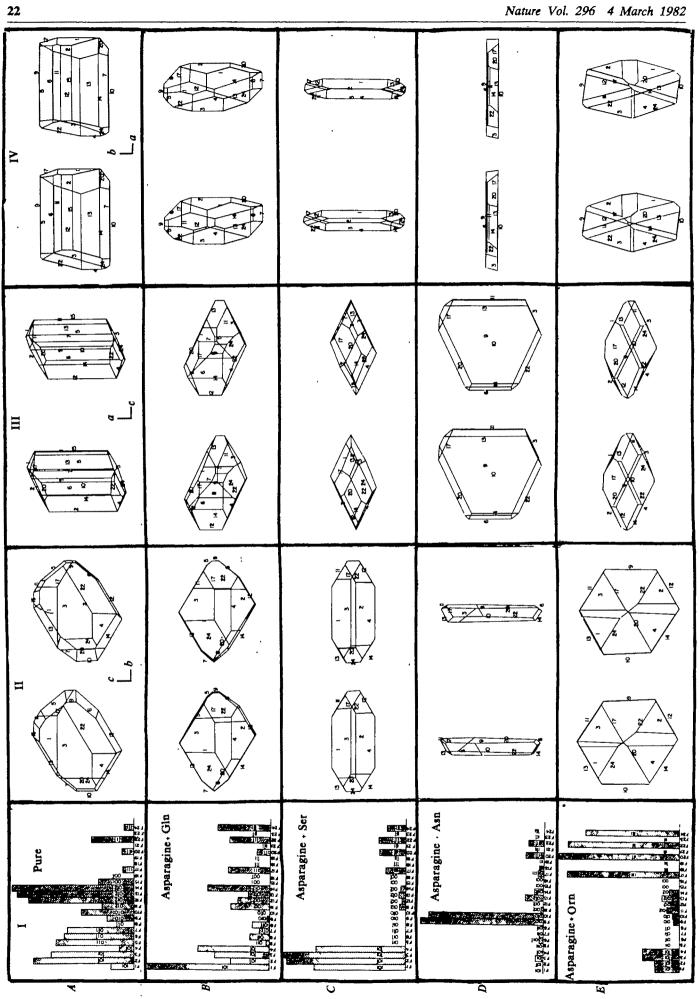


Fig. 2 A comparison between crystals of L-Asn. H₂O grown in presence of various additives: A, none; B, L-Asn+L-Gln; C, L-Asn+L-Ser; D, L-Asn+L-Asp; E, L-Asn+L-Orn. (I) are histograms describing the areas of the faces. The symbols F1, F2 ... correspond to faces 1, 2... denoted on the crystal pictures. The hkl Miller indices of these faces are shown on the histogram. (II), (III) and (IV) are computer-drawn stereographic pictures of the crystals viewed, respectively, along axes a, b and c, which form a right-handed system.

mechanism of the additive. We shall examine the stereochemical relationship between the additive and the crystal structure of the substrate, in order to exploit this knowledge for controlling crystal morphology. The conglomerates of D, L asparagine monohydrate, [D, L-Asn . H_2O] D, L-threonine [D, L-Thr] and D, L-glutamic acid hydrochloride [D, L-Glu . HCl] were selected for study.

Results

D, L-Asn crystallizes from neutral aqueous solutions in the form of a conglomerate [D+L] Asn. H₂O of space group P2₁2₁2₁ (refs 15-17). The crystals show a prismatic morphology with 18 well-developed faces (Figs 1a, 2A). We have found that resolved glutamic acid (Glu), aspartic acid (Asp), glutamine (Gln), serine (Ser), lysine (Lys), ornithine (Orn) and histidine (His) are effective in the kinetic resolution of racemic Asn; these same impurities were selected for morphological studies. Various batches of 100% supersaturated solutions (300 mg per 3 cm³) of D, L-Asn in water were prepared by cooling to room temperature hot filtered solutions, to which resolved impurity (L or D, 5-50 mg) had been added. Crystallization could be accomplished either spontaneously on standing for 1 or 2 days or on seeding with a powder of D, L crystals. In all cases crystals with a normal morphology precipitate first, but after a while (hours or days depending on the efficiency and concentration of the impurity) there appear crystals of distinctly different habit. The precipitates are then separated by decantation, dried and examined (Fig. 1). The crystals of changed and unchanged morphologies have identical internal structure, as checked by both powder and single crystal Weissenberg diffraction photographs, but consist of only L and D enantiomers, respectively, when L impurity is used (and vice versa when D impurity is used) (some typical results are reported in Table 1).

A decrease in concentration of the impurity (from 50 to 5 mg per 3 cm³) results in a progressive decrease in the modification of the affected enantiomer's morphology.

HPLC measurements, carried out in conditions in which Dand L-amino acids are resolved^{18,19}, confirmed that in the crystallization of pure L-Asn in the presence of racemic impurity (D', L'), L' is absorbed preferentially by the L crystals. Molar ratios of L'/D' from 6:1 for D, L-Glu to 30:1 for D, L-Asp were found²⁰.

To check the distribution of the D' and L' impurities in the L crystal grown in this way, single crystals (3–5 mg) were subjected to progressive dissolution in pure water, and the various fractions were analysed by HPLC. While the D' impurity was found only in the first dissolution fractions from L-Asn crystals, L' impurity was present in all fractions at approximately constant concentration. These results strongly support the idea that the impurity is situated only at the outer surface of the substrate crystals of opposite chirality, whereas it is occluded throughout the bulk of the crystal of the same chirality. Clearly the stereochemical similarity between L and L' is the key to the resolution process and to the morphological changes.

To try to clarify the mechanism of the adsorption process, we measured the dimensions, including the areas of all faces, of affected and unaffected crystals, and thus established which growth directions and the areas of which faces are modified by the occlusion of the impurity scheme (1). The crystal dimensions were determined on a diffractometer by measuring the perpendicular distance of each face from a convenient reference point²¹. These measurements were used to derive the coordinates of the bounding corners of the crystal from which the areas of the faces were calculated and the stereographic pictures of the crystals drawn.

Table 1 Morphological separation of enantiomers of D, L-Asn crystallized in the presence of additives*

Additive		Absolute		Enantiomeric
(mg)	Morphology	configuration	$[\alpha]_{\mathbf{D}}$	purity (%)†
L-Asp	Unchanged	D	-28	91
(10)	Changed	L	+31	100
D-Asp	Changed	D	-23.6	78
(20)	Unchanged	L	+28	92
L-Glu	Unchanged	D	-28.6	94
(20)	Changed	L	+29.4	97
D-Glu	Changed	D	-23.5	84
(50)	Unchanged	L	+27.6	91
L-Ser	Unchanged	D	-29.7	98
(25)	Changed	L	+28.5	94
L-Gln	Unchanged	D	-29.7	98
(50)	Changed	L	+28	92
L-Lys	Unchanged	D	-24.4	80
(25)	Changed	L	+26	86
L-His	Unchanged	D	-18.5	61
(50)	Changed	L	+28	92
L-Orn	Unchanged	D	-27	89
(25)	Changed	L	+26.5	87

^{*} From aqueous solutions of concentration 300 mg per 3 cm³.

Figure 2 presents histograms of the measured areas for all the faces of typical crystals of pure L-Asn, L-Asn+L-Gln, L-Asn+L-Ser, L-Asn+L-Asp, and L-Asn+L-Orn, together with computer-drawn stereoscopic pictures of the crystals. Comparison of the histograms shows that, for the impurities L-Ser and L-Gln (Fig. 2B, C), the faces whose areas are relatively enhanced are {101} and, to a smaller extent, {111}, whereas the {011} and {012} faces are reduced in size. L-Glu, L-Orn, L-Lys and L-His induce similar morphological changes but, for the last three (Fig. 2E), the {111} faces are more developed than {101}. L-Asp, in contradistinction, causes L-Asn crystals to assume a completely different platelet-like morphology with predominant faces {010} (see Fig. 2D).

The influence of impurities on the two substrates (2a) and (2b) are described below.

D, L-Glu. HCl crystallizes in space group P2₁2₁2₁ (refs 22, 23) exhibiting a morphology depicted in scheme (2a). Crystallization experiments were performed by dissolving 1 g D, L-Glu. H₂O in 5M HCl (5 cm³) together with variable amounts of resolved L' impurity, filtering the hot solutions and cooling to room temperature (100% supersaturation).

Addition of small amounts (0.5 mg) of seed crystals of racemic Glu. HCl to the cooled supersaturated solution resulted in the initial separation of big crystals of D-Glu. HCl with a morphology as in Fig. 3a. In a second stage (after hours or days depending on the efficiency and concentration of the impurity)

[†] Samples ranging from 70 to 80 mg in weight.

there appeared crystalline powder composed of the L-enantiomer (Fig. 3f). On the basis of resolution power and morphological change the effective impurities may be divided into two groups, one consisting of the basic amino acids L-Lys . HCl, L-Orn . HCl and L-His . HCl, and the other of L-Cys . HCl, L-Thr and L-Ser. The former group, when used in high concentration (above 200 mg per 5 cm3 for His, 100 mg per 5 cm3 for Lys and Orn), strongly depresses the growth of L-Glu. HCl crystals which, however, eventually separate out in powder form (Fig. 3e). When Orn, Lys and His are added in concentrations as low as 10-25 mg per 15 cm3, morphological resolutions can still be performed successfully, the L enantiomer crystallizing in the form of thin $\{001\}$ platelets (Fig. 3b-d). The second group of impurities, Cys. HCl, Thr, Ser, are effective to a lesser extent: the maximal morphological change consists only of a decrease of the thickness of the {001} crystal plates. HPLC experiments, performed as for Asn, showed preferential adsorption on L-Glu . HCl of L' impurity which again proved to be present throughout the bulk of the crystal at a uniform concentration (0.5), while D' is present only in the surface layers of the crystal.

D, L-Thr also crystallizes in space group P2₁2₁2₁ (ref. 24) with a morphology as shown in scheme (2b). The most effective tested impurities in the case of Thr are resolved Glu, Asp, Asn and Gln. The crystals obtained from 100% supersaturated filtered solutions (900 mg D, L-Thr in 5 cm3 pure water), in the presence of these impurities, appear in the form of bars coated with powder (Fig. 3b). As the crystals twin easily, morphological studies were best performed by separate crystallization of L-Thr in the presence of L' and D' impurities, performed in parallel in identical conditions. Photographs of crystals of pure L-Thr (Fig. 3g) and of L-Thr crystallized in the presence of increasing amounts of L-Glu (Fig. 3h-j) show gradual morphological modification. On the other hand the morphology of L-Thr is left unchanged by the presence of the same amounts of D-Glu. The impurities mainly affect the growth of the {210} faces of the bar-like crystals, turning these crystals first into thin needles and eventually into powder.

Discussion

Having characterized the morphological changes in terms of specific directions of crystal growth we shall now investigate whether we can obtain information about the situation of the occluded molecule in the substrate lattice, and the associated question of the correlation of the stereochemistry of the impurity molecule with its morphological influence.

We note that well-developed faces arise from strong attractive forces between molecules forming a layer parallel to the face. The tendency for growth in a certain direction depends on the attachment energy between the layer of molecules on the crystal face and the rest of the crystal²⁵. The habit changes indicate that introduction of the impurity perturbs the layer and the attachment energies for the various faces to different extents.

In all three systems $\operatorname{Asn.H_2O}$, $\operatorname{Glu.HCl}$ and Thr , the effective impurity molecules have an arrangement of the carboxyl and amino groups directly attached to the asymmetric carbon identical to that of the host, but with different side chains. In crystals of all three substrates (Figs 4–6) the amino and carboxyl groups attached to the asymmetric carbon atom participate in several H-bonds. We expect that, in the 'doped' crystals, the H-bonding groups of the occluded impurities will occupy the same positions as do the corresponding groups of their host substrates, as they supply equivalent strong interactions. Thus the side chains of the impurities replace those of the substrates, but introduce contacts which differ from those of the host molecule. We shall account for the changes in crystal habit in terms of the effects of the contacts made by the impurity side chain at various crystal faces.

In the crystal structure L-Glu . HCl each side chain is directed approximately along the c-axis and makes H-bonds in this direction (Fig. 4). Thus it is clear why impurities such as L-Lys . HCl, L-Orn . HCl, L-His . HCl, L-Thr and L-Ser inhibit growth along the c-direction, decreasing the thickness of the $\{001\}$ plates of the crystal.

We first examine the striking morphological modification brought about by L-Asp on L-Asn, where the essential structural change at the site of the impurity is the removal of one H-bonding amide H atom of Asn. Contrary to other additives, which are found in the bulk of the substrate crystal in amounts ranging from 0.5 to 1%, up to 12% of L-Asp has been found in single crystals of L-Asn, indicating formation of solid solution between additive and host. We therefore measured the cell dimensions of several pure and affected L-Asn crystals (four of each). The average cell dimensions of pure L-Asn are a =5.582(2), b = 9.813(3), c = 11.791(4)Å, those of L-Asn + 10% L-Asp are a = 5.567(3), b = 9.835(5), c = 11.785(3)Å. These axial changes are compatible with the occluded Asp having its carboxy group in a cis rather than trans configuration. (There is much literature evidence in favour of the cis O-H bond. The trans O-H bond has not been found, with one exception²⁶, when O-H participates in an intramolecular O-H···O bond²⁷.) The shortening along a of 0.015 Å is in keeping with replacement of the N-H(cis)---O bond, of length 2.94 Å and directed with a

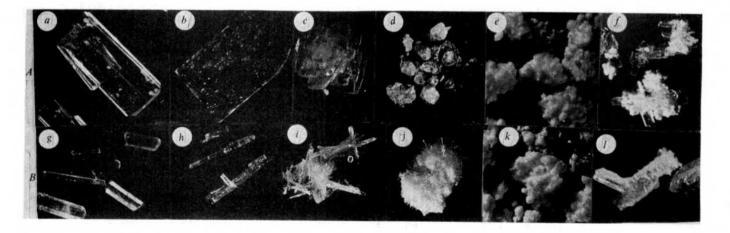
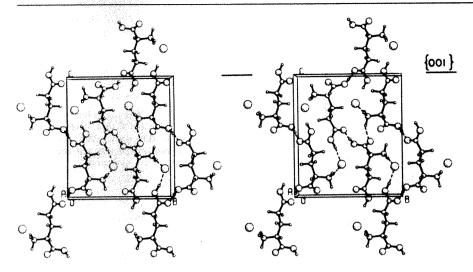


Fig. 3 A, Crystals of L-Glu. HCl grown in the presence of increasing amounts of impurity: a, none or D-Glu+L-Lys; b, +2 mg cm⁻³ L-Lys; c, +5 mg cm⁻³ L-Lys; d, +10 mg cm⁻³ L-Lys; e, +50 mg cm⁻³ L-Lys. f, Crystals of D, L-Glu. HCl grown in the presence of L-Lys. The plates are the D-enantiomer while the powder is the L-enantiomer. B, Crystals of L-Thr grown in the presence of different amounts of L-Glu; g, none or D-Thr+L-Glu; h, +2.5 mg cm⁻³ L-Glu; i, +7.5 mg cm⁻³ L-Glu; j, +15 mg cm⁻³ L-Glu; k, +30 mg cm⁻³ L-Glu. l, Crystals of D, L-Thr grown in the presence of L-Glu. The bars are the D-enantiomer while the powder is the L-enantiomer.



A

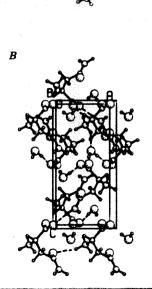
Fig. 4 Stereographic packing diagrams for L-glutamic acid. HCl as viewed along the a-axis.

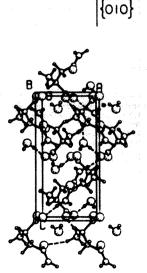
large component along a (Fig. 5), by an OH(cis)···O bond which tends to be 2.6 Å in length. The increased length of the b axis of 0.022 Å is in keeping with a replacement of the N-H(trans)···O bond, of length 3.05 Å and directed mainly along b (Fig. 5A), by an O···O contact which may be as long as 3.5 Å; this O···O interaction is repulsive because the lone pair electrons of these two atoms are directed towards each other²⁶⁻²⁹. Indeed these two new interactions introduced by the impurity, namely the attractive O-H···O and the repulsive O···O ones, may explain the formation of $\{010\}$ plates of the affected crystal. The $\{010\}$ layer energy is enhanced by the former, while the $\{010\}$ attach-

ment energy is severely reduced by the repulsive O···O interaction, thus developing large {010} faces.

The impurities L-Gln, L-Glu, L-Orn, L-Lys, L-His and L-Ser on L-Asn all have the same general effect: the preferred crystal growth along the a direction is replaced by a tendency for growth along b, and concomitantly with a reduction in area of the $\{011\}$ faces. We may rationalize these morphological changes as follows. First, the amide N-H(cis).--O (carboxy) and amino N-H---O (amide) bonds, which are directed mainly along the a-axis (see Fig. 5B) would be absent at the site of impurity, thus inhibiting growth along a. Furthermore the Asn molecules

Fig. 5 Stereographic packing diagrams for L-asparagine . H₂O. A, view along the a-axis. Here one asparagine molecule is replaced by an aspartic acid molecule (filled circles). Some of the crystal faces affected by the impurities are indicated. B, view along the b-axis.





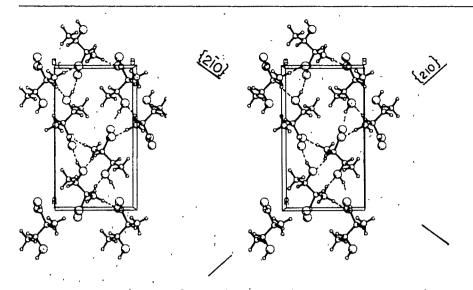


Fig. 6 Stereographic packing diagram for L-threonine as seen along the c-axis. The directions parallel to the affected faces are indicated.

related by the a translation form the highest number of contacts per pair of molecules, and thus growth along this direction should be the most affected. The relative increase of growth along b for affected crystals may be appreciated when we note that only one amide N-H--O bond directed almost along b would be absent at the site of the impurity.

The rupture of the OH---O bond of the side chain hydroxyl of the substrate Thr at the site of the impurity would certainly inhibit growth of the {210} faces perpendicular to the needle c-axis, as the O-H---O bonds are directed almost parallel or perpendicular to these faces, thus lowering both their layer and attachment energies (Fig. 6). However, the growth along the needle c-axis should not be dampened. Thus the observed effect of impurities, including thinner needles and eventually powder, is compatible with such a model.

Conclusions

On the basis of all our data, we suggest a mechanism involving two steps:

(1) Binding: the strong interactions between the amino acid group NH₃+-CHCOO of the substrate and the molecules at the growing site lead to binding of the impurity molecule at the surface of the crystal, in the same way as a substrate molecule is bound. A high degree of stereospecificity is needed to fit the chiral additive to the structured surface, and this explains the L'/D' selectivity of the effect.

(2) Inhibition: once the impurity has been adsorbed at the surface, it disturbs the regular deposition of the next layer of substrate molecules in the specific direction along which the modified side chain group emerges. The obstacle is eventually removed by overgrowth resulting in the inclusion of the foreign material in the bulk of the crystal. This mechanism would rationalize the different inhibition power of the various impurities, in terms of the hydrogen bonding and acid-base properties of the side chains of these molecules (for example, the strong effect of L-Lys, L-His and L-Orn on L-Glu. HCl).

It is instructive to consider the implication of this mechanism for crystals containing a polar b axis. Here the faces (hkl) and $(h\bar{k}l)$ have different structures³⁰⁻³³. Thus their growths can be affected differentially by an appropriate 'tailor-made' impurity. The specific morphological changes induced would then allow us to determine the absolute configuration of the substrate akin to the Bijvoet method. Studies in these directions on the systems L-Lys. HCl, and (L) cinnamoyl alanine, have been successful and are reported in the following paper³⁴

Finally, apart from providing us with a method for controlled modification of crystal morphology, this work allows a deeper - understanding of the role of the tailor-made impurity thus strengthening the empirical approach for assignment of relative configuration we proposed previously¹⁰, it also enables us to select better additives for efficient kinetic large scale resolution of conglomerates, and for growing single crystals of interest for material science and crystallographic measurements.

Parallel calculations of intermolecular interactions presently in progress, using existing techniques35, have already provided a quantitative basis for the mechanism proposed above, for simple systems such as benzamide-benzoic acid (Z.B.-Y., L.A., M. Idelson, M.L. and L.L. work in preparation).

We thank the Volkswagen Stiftung and the US/Israel Binational Fund for financial support, and Professor M. D. Cohen for critical reading of the manuscript.

Received 27 May, accepted 9 December 1981

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Absolute configuration of chiral polar crystals

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A method is described for direct determination of absolute configuration of chiral polar crystals, based on changes in crystal habit induced by tailor-made impurities. The method embodies the assignment of the direction of the chiral substrate molecule with respect to the crystal polar axis based on differences in the hemihedral faces of the substrate crystals as grown from solution in the presence and absence of impurities. The molecular packing requirements and the choice of impurities for application of this method in lysine and trans-cinnamoyl alanine are outlined.

THE configuration of chiral molecules had been defined only relatively, until 1951 when Bijvoet applied the method of anomalous dispersion of X rays to crystals of ammonium rubidium tartrate, thus placing stereochemistry on an absolute scale¹. In 1973 it was shown that two other, independent methods, noble gas ion reflection mass-spectrometry² and circular dichroism³⁻⁵, give chirality assignments which are in accord with the assignments according to Bijvoet (see review in ref. 6).

The assignment of the absolute configuration of chiral molecules in chiral crystals which develop hemihedral faces has long challenged the chemical crystallographer. In principle any method which establishes the orientation of a given functional group of the molecule with respect to the crystal axes or to a hemihedral face bounding the crystal, fixes the absolute configuration of the molecule. Several attempts have been made to deduce absolute configuration from crystal habit. Waser⁷ tried to establish the absolute configuration of D-tartaric acid by correlating the relative rates of growth of the hemihedral (hkl) and (hkl) crystal faces with the ease of attachment of the 'free' molecule at either face. However, Bijvoet⁶ later showed Waser's conclusions to be incorrect. Indeed, Turner and Lonsdale⁸ had earlier criticized Waser's reasoning, arguing that, within the limits of his assumptions, the ease of approach at either face is identical. In 1949 Wells examined the differences in the habit and growth directions of the polar crystals of resorcinol grown from various solvents. He interpreted the solvent effects as being due to the different interactions of the solvent with the different exposed faces of the crystal, which have polar OH groups at one end and hydrophobic benzene groups on the other. Booth and Bukley10 proposed that the same kind of interactions may be responsible for the growth behaviour of ethylenediamine tartrate (monohydrate) crystals, pure or in the presence of boric acid. Paul, Curtin and co-workers^{11,12} recently correlated the direction of the unique polar axis of p-bromobenzoic acid anhydride crystal with that of the preferential attack of ammonia gas on appropriate faces of single crystals of this compound.

Our recent studies on morphological changes in organic crystals as induced by 'tailor-made' impurities¹³ suggested a new direct method for the assignment of absolute configuration of chiral crystals and molecules. This method embodies the establishment of the orientation of the chiral molecule with respect to the crystal polar axes.

Crystal morphology change and nature of added impurity

Kinetic resolution of a large variety of racemic conglomerates in the presence of tailor-made impurities revealed, in many systems, stereoselective adsorption of the impurity at the surface of one enantiomorph, followed by drastic changes in its crystal habit, while the other enantiomorph remains unchanged¹⁴. Through these habit changes, a stereochemical correlation has been established between the molecular structure of the

impurity, the crystal structure of the substrate, and the affected growth directions.

A detailed study of such morphological changes induced in the crystals of L-asparagine. H₂O, L-threonine and L-glutamic acid.HCl, by other L-amino acids used as additives, led to the proposal of a two-step mechanism of binding and inhibition¹³. By virtue of strong attractive interactions (H-bonds, ionic) the unmodified (with respect to substrate molecules) amino acid group of the impurity binds stereospecifically at a site suited to the amino acid group of the substrate molecule on the surface of the host crystal. However, the modified chain of the occluded impurity perturbs the regular deposition of the oncoming layers of the crystal in specific directions, as defined by the hydrogen bonding pattern of the side chain of the substrate. The rate of growth in these directions is thus decreased, leading to changes in crystal morphology. These results, which have been extended to other hydrogen bonded systems (Z.B.-Y., L.A., M.L. and L.L., work in preparation), suggested that crystal habit can be modified in a controlled way by use of appropriate crystal growth inhibitors.

We have applied this concept to polar crystals, racemates and meso compounds to design a new direct method for the assignment of absolute configuration of chiral molecules. Although here we consider only polar crystals. We shall first present a topological model which outlines the requirements for assignment of absolute configuration, and then describe its successful application to Lys.HCl.2H₂O and trans-cinnamoyl-alanine.

Modelling impurity effects on polar crystal growth

We consider a chiral molecule (or a non-chiral molecule adopting a chiral conformation), represented schematically as X-A, packing in a polar crystal whose unique polar axis is parallel to the X-A molecular axis. These requirements can be fulfilled in all chiral polar space groups, among which are P1, P2₁, C2, and

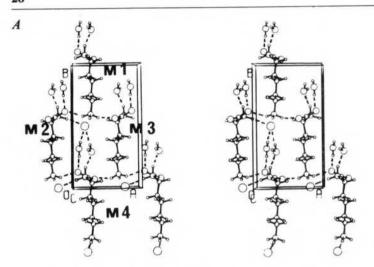


Fig. 1 Packing arrangement of the crystal structure of Llysine \cdot HCl \cdot 2H₂O viewed along the $_{c}$ C-axis. A, stereographic view; B, packing arrangement as delineated by the observed hk0crystal faces.

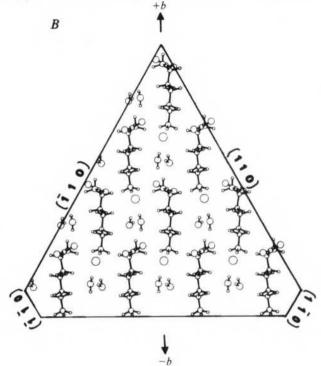
 $P3_1$. In principle the approach may be extended to any chiral crystal (such as P222, $P2_12_12_1$), in which the molecule X-A is oriented in a general polar direction.

The crystal model is depicted in scheme (1) for a structure appearing in the common polar space group P21. This crystal is delineated by faces f1, f2 in the +b-direction and f3, f4, f5 in the b-direction. By virtue of the crystal (point) symmetry the faces within each pair (f1, f2) and (f3, f5) are related by 2-fold symmetry. The polar nature of the crystal precludes a symmetry relationship between the +b and -b directions. Thus faces within the pairs (f_1, f_5) and (f_2, f_3) cannot be related by symmetry. Consider that in this crystal only A groups emerge at faces f1 and f₂ whereas X groups emerge at faces f₃, f₄, f₅. The essence of the problem is that conventional X-ray diffraction does not provide information as to whether the molecule X-A points in the +b or in the -b direction. In other words, it does not allow one to distinguish between the real crystal scheme (1a) and the hypothetical enantiomeric structure in which the orientation of X-A with respect to the b-axis is opposite scheme (1b).

Applying the two-stage mechanism of adsorption-inhibition, described above, appropriate impurities X-Y or Z-A should influence asymmetrically the growth rate of the crystal along the polar b-axis, thus allowing one to determine whether X-A is directed along the +b- or -b-direction, through selective morphological changes. An impurity X-Y will bind to faces f1 and f2, but not to f3, f4, f5, and once bound, will hinder growth along the +b and possibly other directions, but not along -b in the real crystal of scheme (1) (the opposite would happen in the crystal of scheme (1b)). We would then predict changes in crystal habit involving either an increase of the areas of faces f₁ and f₂ relative to those of other faces of the crystal, or the development of appropriate, additional new faces. The same logic applies to an impurity Z-A which we predict would affect growth of faces f₃, f₄, f₅. The morphological differences between crystals grown in the presence and in the absence of the impurity would then indicate the direction of the modified group in the given crystal, thus establishing the direction of the substrate molecule X-A with respect to the polar axis. Consequently the absolute configuration of the crystal and of the chiral molecular constituents can be derived. Note that the additive need not be chiral and, even if it is, the assignment of absolute configuration of the crystal does not require a knowledge of the absolute configuration of the additive.

Results

(L)Lys.HCl crystallizes from water as a dihydrate in a monoclinic structure $^{15-17}$ of space group P2₁ with Z=2 and a=7.49,



b=13.32, c=5.88 Å, $\beta=97.50^\circ$. The packing arrangement viewed along the c-axis is shown in Fig. 1. The Lys molecules are oriented with respect to the b-axis so that the ε -NH $_3^+$ group is directed along -b, and the amino acid function (NH $_3^+$ C(CO $_2^-$) along +b. Crystals of (L)Lys.HCl.2H $_2$ O grown from water (900 mg cm $_3^-$), cooling in the presence of seeds, 0.5 mg), appear mainly as prisms with an almost triangular section, with the b-axis bisecting the triangle and c directed along the long axis of the prisms (Figs 1B, 2A). The orientations of the molecules relative to the observed hk0 crystal faces are shown in Fig. 1B.

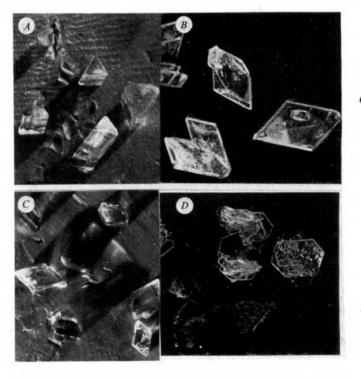


Fig. 2 Crystals of L-lysine.HCl.2H₂O grown in the absence and presence of impurities: A, pure; B, +additive L-Orn; C, +L-NorVal; D, +L-MeLys.

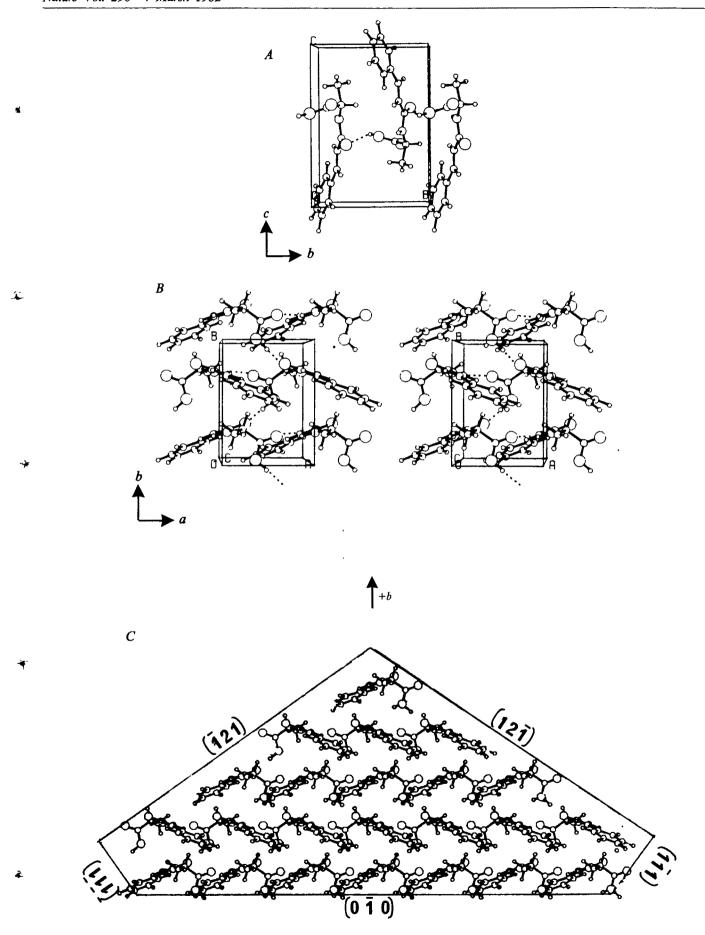


Fig. 3 Packing arrangement of trans-cinnamoyl-L-alanine: A, viewed along the a-axis; B, viewed along the c-axis; C, viewed along the c-axis as delineated by the observed (hk0) crystal faces.

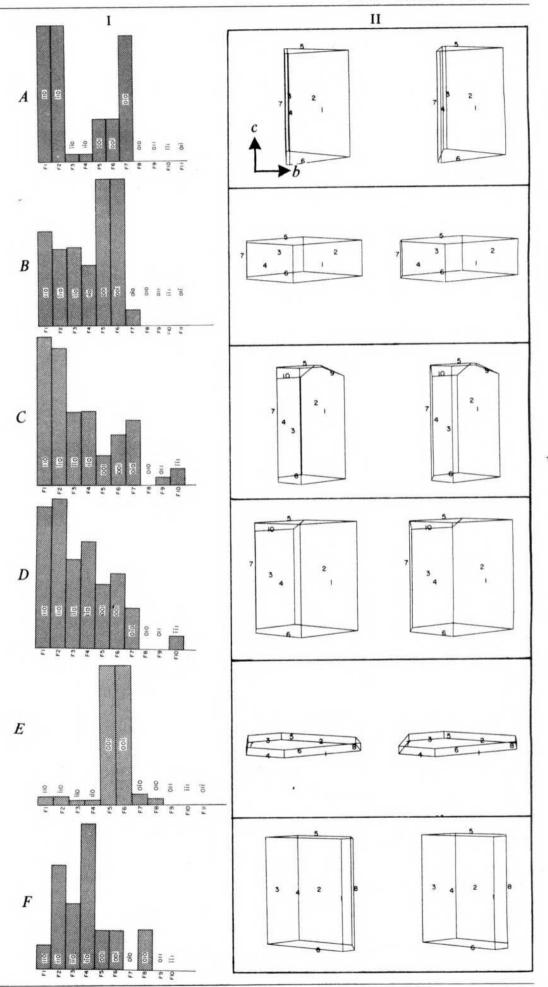
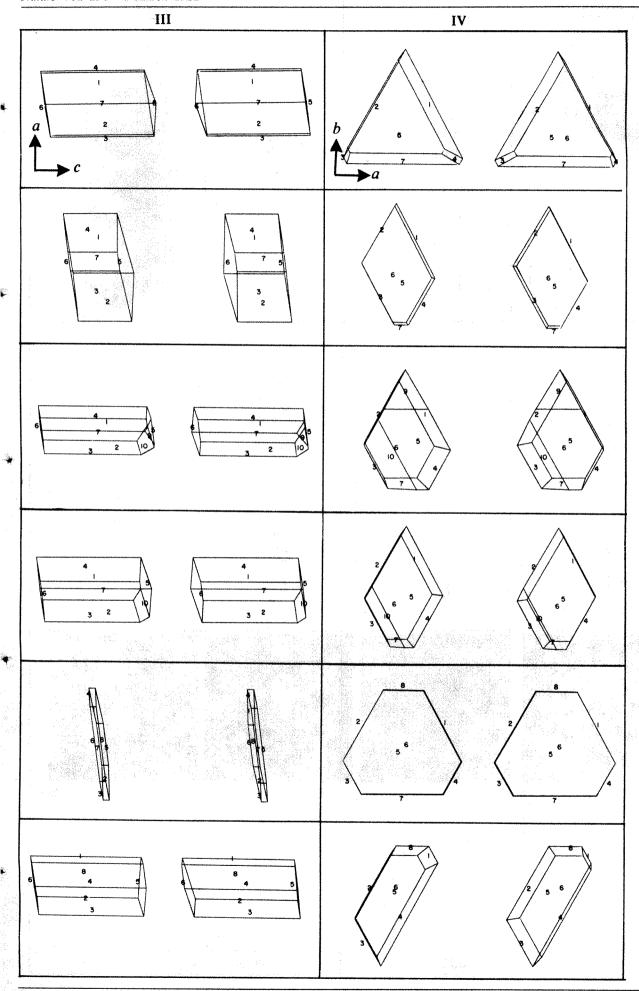


Fig. 4 A comparison between crystals of L-lysine.HCl.2H₂O grown in the absence and presence of additives: A, pure; B, +L-Orn; C, +L-NorVal; D, +L-NorLeu; E, +L-MeLys; F, +ε-aminocaproic acid. I, Histograms describing the areas of the faces. II, III and IV, computer-drawn stereographic pictures of the crystals viewed along axes a, b and c respectively.



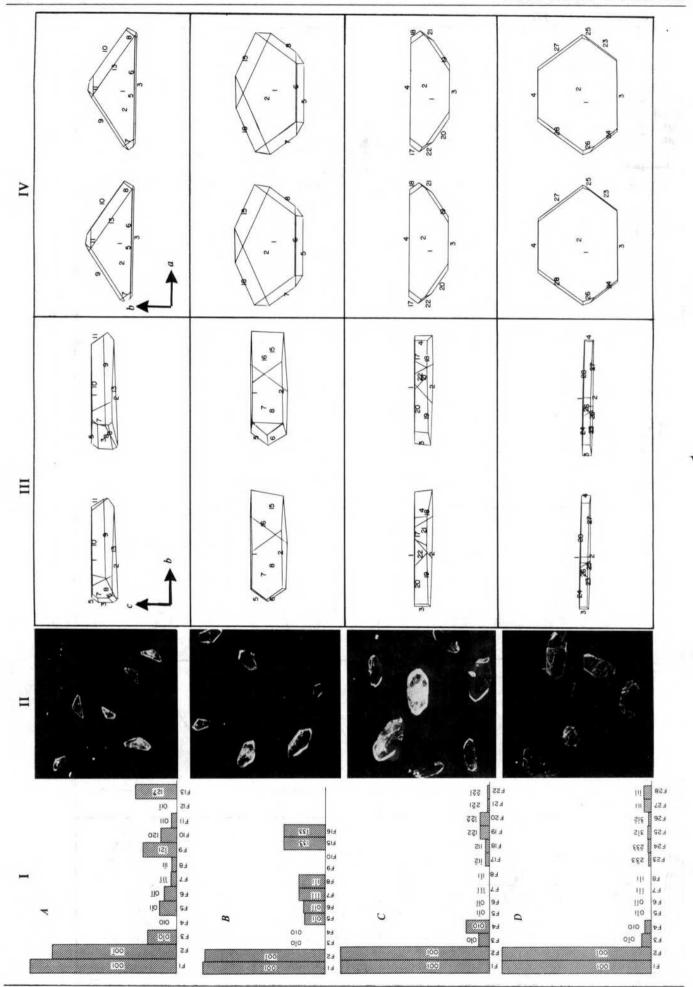


Fig. 5 A comparison between crystals of cinnamoyl L-alanine grown in the absence and presence of additives A, pure; B, +3% methyl ester; C, + trans-cinnamoyl (D)-alanine; D, +trans-cinnamoyl D-serine. I, Histograms describing the areas of the faces. II, Photographs of crystals. III and IV, Computer-drawn stereographic pictures of the crystals viewed along axes a and c respectively.

Bearing in mind the above conclusions, the crystal structure suggested that an impurity with modified carboxyl or α -amino groups will disturb growth along +b but not along -b, whereas modifications in the side chain will, by contrast, disturb growth along -b, but not +b. For both cases we may expect some modification in the a and c directions. (1) L-methyl lysinate (MeLys) and ε -aminocaproic acid (which is non-chiral) and (2) L-ornithine (Orn), L-norleucine (NorLeu), and L-norvaline (NorVal) were selected as additives representative of the two cases. Crystals of L-Lys.HCl.2H2O, were grown from water in the presence of each impurity separately. The concentrations of the additives in solution were: L-Orn.HCl: 200 mg cm⁻³; L-NorLeu: 10 mg cm⁻³; L-NorVal: 10 mg cm⁻³; L-MeLys.HCl: 50 mg cm⁻³; ε -aminocaproic acid: 200 mg cm⁻³. The amounts of additives used in solution were chosen on the basis of the respective solubilities in H₂O, and are always much larger than the amounts found in the bulk of the substrate crystals after crystallization.

The crystals obtained were separated by decantation, dried and examined (Fig. 2B-D). The faces were characterized and the dimensions measured as described previously¹³. The computer-drawn pictures of pure and affected crystals are shown in Fig. 4 together with the histograms of the face areas. The additives L-Orn, L-NorLeu, and L-NorVal inhibit growth along the -b-direction, as indicated by the pronounced increase in the areas of the symmetry-related (110) and (110) faces (see Figs 2B, C and 4B, C, D). This change in crystal habit is primarily a consequence of rupture at the impurity site of the two (ε -amino)N-H···O(carboxyl) H-bonds linking molecule M(1) to M(2) and M(3), related to it through 2_1 -axes (see Fig. 1A). The H-bonded system (ε -amino)N-H···Ck HOH along

The impurity L-MeLys affects growth along the +b-direction inducing formation of the (010) face. Although we could not pinpoint the precise orientation of the O-CH₃ group of the impurity, there can be little doubt that this group would disrupt the H-bonded network along the +b-direction because the substrate CO₂ group participates in several H-bonds along +b. from molecule M(4) to M(1) and M(3) and to two water molecules (Fig. 1). MeLys also affects growth along the c-axis, turning the crystal into (001) plates (Figs 2D and 4E). This change is not surprising in view of a possible rupture of the $(\varepsilon$ -amino)N-H···O(carboxyl) bond interlinking lysine molecules along the c-axis and of the steric hindrance induced by the methyl group of MeLys along the short c-axis. ε -Aminocaproic acid induces formation of the (010) crystal face and thus has a similar effect to MeLys, which may be explained on analogous lines. However, unlike MeLys, this impurity does not affect growth along the c-axis, presumably because no steric hindrance is induced along this direction.

Trans-cinnamoyl-L-alanine

the -b-direction would also be hindered.

Trans-cinnamoyl-L-alanine crystallizes from CHCl₃, MeOH or EtOH in polar crystals of space group P2₁, with a=6.1, b=8.2, c=11.3 Å, $\beta=90.6^{\circ}$. In the crystal^{18,19} (Fig. 3) the molecules are oriented such that the carboxyl O-H participates in an O-H···O(amide) bond whose vector has a major component along the -b-direction, and the C(chiral)-H bond is almost parallel to +b (Fig. 3A). Pure trans-cinnamoyl-L-alanine crystals, grown from CHCl₃ by slow evaporation at room temperature, appear as thin pentagonal (001) platelets, with the symmetry axis b bisecting the pentagon perpendicular to its base (Figs 3C, 5A). Figure 3C shows the orientation of the molecule with reference to crystal faces in the pure crystal. Following the above analysis, the methyl ester of the acid should decrease the

growth rate in the -b-direction. By the same reasoning growth should be inhibited along the opposite direction, +b, using trans-cinnamoyl-D-alanine or trans-cinnamoyl-D-serine as the impurity: binding at the surface of the substrate crystals should be possible for such impurities by virtue of strong H-bonds, but once bound their C(chiral)—CH₃ [or C(chiral)—CH₂OH] group would replace the C(chiral)—H group of the substrate thus hindering growth along the +b-direction. Such a conformation of the impurity molecule would not incur intramolecular steric contacts. Indeed the ease of such an effective interchange of the CH₃ and H substituents at the chiral C atom is demonstrated in the crystal structure of o-chlorocinnamoyl-L-alanine^{18,19}.

Crystals were grown from CHCl₃ in the presence of increasing amounts (1, 3, 5 and 10%) by weight) of these three impurities, in the same conditions as for the pure compounds. The morphologies of these crystals are presented (Fig. 5). The most pronounced change induced by the methyl ester of transcinnamoyl-L-alanine is the large increase in the areas of the symmetry-related $(1\overline{1}1)$ and $(\overline{1}\overline{1}\overline{1})$ faces. Other changes are the formation of the $(0\overline{1}1)$ and $(0\overline{1}\overline{1})$ faces instead of $(0\overline{1}0)$ and replacement of the steep $(\overline{1}21)$ and (120) faces by the shallower $(13\overline{3})$ and $(\overline{1}33)$ faces. The relative increase in areas of these faces is, by and large, proportional to the amount of impurity in solution (1-5%).

We account for the large increase in the area of the $(1\bar{1}1)$ face in terms of the observation that the (carboxyl)O-H···O(amide) H-bond emerges from this face almost perpendicular to it, making an angle of 16° with its normal. Thus the replacement of the substrate group by O-CH₃ at the site of the impurity precludes formation of the O-H···O H-bond and hinders the natural growth perpendicular to the $(1\bar{1}1)$ face, with a subsequent increase in its area.

The impurity trans-cinnamoyl-D-alanine induces formation of the (010) face as shown in Fig. 5 C. This morphological change reflects an inhibition of growth along the +b-direction, as predicted above. A similar morphological change is induced by the additive trans-cinnamoyl-D-serine (Fig. 5D). In the case of trans-cinnamoyl-D-alanine the effect is so pronounced that the (010) face, absent in the pure crystal, is much more developed than the naturally developed (010) face.

Discussion

The experimental findings on lysine and cinnamoyl alanine indicate that it is possible through modification of crystal morphology by tailor-made impurities in appropriate systems, to establish the absolute configuration of the substrate molecule. The present approach differs radically from that of Waser, who tried to ascertain absolute configuration on the basis of the morphology of the pure crystal by comparing the relative ease of attachment of molecules to hemihedral faces $\{hkl\}$ and $\{hkl\}$. The latter approach is full of pitfalls, because the sole difference between the energy of attachment²⁰ to the two faces arises from solvent effects and differences in polarizability and conformation between the crystal-bound molecule at the surface and the to-be-attached 'free' molecule. Effects of these kinds are difficult to quantify. These uncertainties do not affect our approach because the change in crystal habit originates essentially from the difference in the ease of approach of the to-beattached molecule to the same face in the poisoned and unpoisoned crystals. Moreover, the reliability of the method is increased by observing a progressive change in crystal habit as a result of increasing concentration of impurity in solution, and by a cross-check of the assignment by using various impurities, appropriately designed, so that they will affect stereoselectively either polar growth direction.

The method is not limited to chiral crystals, but is applicable also to racemic or meso compounds, with certain desired

packing features, for assignment of the absolute configuration of the chiral additive. This extension is based on the fact that, in contradistinction to chiral crystals, in racemic compounds comprising chiral R and S moieties, one can specify unambiguously the chirality of the moiety which emerges from a given crystal face. Crystallization of such a racemic crystal poisoned with chiral impurity may affect enantioselectively one of the pair of enantiotopic crystal faces. From the change in crystal habit it may be possible to fix the absolute configuration of the additive. An analysis of the packing requirements and application of this method to serine-threonine will be presented elsewhere21

The rules of morphological changes, as outlined here, also

Received 27 May, accepted 9 December 1981.

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apply to solid solutions, with the advantage that one may there be able to locate precisely the atomic positions of the occluded guest additive by X-ray diffraction. This will eventually allow us to study these phenomena on a quantitative basis, leading hopefully to a better understanding of the correlation between molecular structure and crystal morphology.

We thank Professor Duilio Arigoni for his prompting (since 1969) that there must be a simple way to extract absolute configuration of chiral molecules from morphology, also Professor M. D. Cohen for discussions and Sarah Ariel for the crystal structure coordinates of cinnamoyl-L-alanine, and the US/Israel Binational Science Foundation, Jerusalem, and the Volkswagen Stiftung Foundation for financial support.

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A monoclonal antibody defining antigenic determinants on subpopulations of mammalian neurones and Trypanosoma cruzi parasites

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An IgM λ class monoclonal antibody raised against membranes from rat dorsal root ganglia defines a novel antigenic determinant expressed by subpopulations of mammalian central and peripheral neurones. In the presence of complement the antibody is cytotoxic to mammalian neurones in vitro. The same antibody labels Trypanosoma cruzi, the protozoan responsible for Chagas' disease. Classes of mammalian neurones and cardiac muscle that are labelled by the antibody are known to degenerate in Chagas' disease. The common neuronal and trypanosomal antigens recognized by the antibody may therefore be important in pathogenic events underlying this disorder.

CHAGAS' DISEASE results from infection with the protozoan Trypanosoma cruzi, affects an estimated 12 million people in Latin America¹ and is characterized by extensive neuronal degeneration. In the peripheral nervous system, there is a massive degeneration of sympathetic², parasympathetic³ and enteric^{4,5} neurones. Lesions of Purkinje neurones in the cerebellar cortex have been reported^{5,6} and there is also significant degeneration of cardiac muscle in both the acute and chronic stages of the disease7.

Although neuropathy does not result from direct parasite invasion, the precise mechanism underlying neuronal degeneration is still unclear. It has been suggested that Chagas' disease may have an immunological basis, consistent with the destruction of cardiac and neuronal tissue by an autoimmune mechanism^{8,9}. One explanation for the origin of this autoreactivity is that the host and parasite share common antigenic determinants, such that the development of anti-parasite immunity allows cross-reactivity with self components.

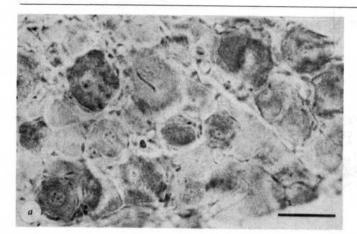
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Previous studies have demonstrated that sera from patients with T. cruzi infection contain antibodies that recognize mammalian vascular endothelial cells¹⁰, components of peripheral nerve, possibly Schwann cells¹¹, the plasma membrane of striated muscle and endothelial cells¹² and certain peripheral and central neurones¹³. However, in these studies the nature of the antigenic stimulus is unknown.

We report here that a monoclonal antibody, generated against mammalian dorsal root ganglia, exhibits a unique specificity for subpopulations of central and peripheral neurones and also recognizes an antigenic determinant on the protozoan T. cruzi. Strikingly, all classes of neurones we have examined that are known to undergo degeneration in Chagas' disease express this antigenic determinant. In addition, cardiac muscle is stained by the antibody whereas skeletal muscle, which is unaffected in Chagas' disease, is not.

Production and characterization of antibodies

With the original aim of generating antibodies that might provide markers for subpopulations of primary sensory neurones, dorsal root ganglia were dissected from 7-day-old CD rats (Charles River Breeding Laboratories). Ganglia pooled from



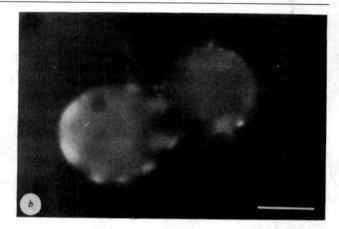


Fig. 1 Immunocytochemical labelling by CE5 of rat dorsal root ganglion neurones in fixed sections and dissociated cell culture. For staining of fixed sections, rats were anaesthetized with ether and perfused via the aorta with 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature. The brain, spinal cord and dorsal root ganglia were removed and placed overnight at 4 °C in 0.1 M phosphate buffer containing 30% w/v sucrose. 30-µm sections were cut with a freezing microtome and incubated in CE5 hybridoma supernatant diluted 1:1 with PBS (pH 7.4) for 18-24 h at 4 °C. Sections were then washed three times with PBS and incubated for 20 min with either horseradish peroxidase- or FITC-conjugated rabbit anti-mouse IgG (Litton Bionetics) diluted 1:25 with PBS. Sections processed for fluorescence were then washed three times in PBS mounted on glass slides, and viewed on a Leitz Ortholux II microscope equipped with epifluorescence illumination. Photographs were taken with 2-min exposures using 400 ASA film. For peroxidase immunocytochemistry, sections were washed three times in 0.05 M Tris-HCl (pH 7.6) after removal of second antibody and incubated with 0.05% diaminobenzidine and 0.002% H₂O₂ in 0.05 M Tris-HCl for 10-20 min. Stained sections were dehydrated in alcohol and xylene, mounted in Permount and viwed under bright field and interference contrast optics. a, Immunoperoxidase staining of sensory neurone cell bodies in sections of cervical dorsal root ganglia. Immunoreaction product is present throughout the cytoplasm although membrane associated staining can also be observed. In many sections, large neuronal cell bodies appeared more densely stained than small neurones. Stained fibres can also be seen within the section although satellite and other non-neuronal cells appear unstained. Scale bar, 50 µm. b. Immunofluorescent staining of rat dorsal root ganglion neuronal cell bodies, grown in dissociated cell culture for 10 days. Dorsal root ganglia were removed from rat embryos at 19 days of gestation, incubated with 0.05% trypsin and 0.01% collagenase (Worthington type IV) for 20 min at 37 °C and dissociated by trituration. Cells were plated onto collagen and fibronectin-coated 35-mm tissue culture dishes in Ham's F12 medium containing 7.5% heat-inactivated rat serum, 4% 17-day rat embryo extract, 2 mM glutamine, essential vitamins, 5,000 U ml⁻¹ penicillin and streptomycin, 44 mM glucose and 1 µg ml⁻¹ purified nerve growth factor. Cultures were fed every 2-3 days and treated with cytosine arabinoside (10-5 M) for 24-28 h soon after plating. Cultures were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, and processed for immunofluorescence as described above. Patchy staining of two sensory neurone cell bodies can be observed with staining apparent in both the cytoplasm and in association with the surface membrane. Scale bar, 20 µm.

six rats were sectioned in a tissue chopper and homogenized in 0.05 M Tris-HCl (pH 7.6) with a Dounce pestle, followed by centrifugation at 2,000g for 10 min. The resulting supernatant was then centrifuged at 50,000g for 30 min and the crude membrane pellet used for immunization.

Six-week-old female BALB/c mice were injected intraperitoneally with 0.2 ml of the membrane suspension (10 mg ml⁻¹) in phosphate-buffered saline (PBS pH 7.4) emulsified 1:1 with Freund's complete adjuvant. Injections were repeated twice at 14-day intervals. Three weeks later a final injection was performed, without adjuvant, and the spleens from immunized mice were removed after a further 3 days. Lymphocytes were fused¹⁴ with X63.Ag 8.653 myeloma cells¹⁵. Hybrid clones were screened after 10 days and positive wells were cloned three times by limiting dilution.

Hybridoma supernatants were screened by indirect immunofluorescence using fluorescein isothiocyanate (FITC)conjugated rabbit anti-mouse IgG on 30-µm transverse sections of the dorsal root ganglia and spinal cord of rats fixed by perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Of 288 hybridoma supernatants examined, 24 exhibited positive staining of neuronal cell bodies and an additional 4 stained radial glial fibres. One supernatant, designated CE5, labelled dorsal root ganglion neurones (Fig. 1a), motoneurone cell bodies (Fig. 2c) and large-diameter neurones within laminae IV and V of the dorsal horn with little or no staining of neuronal cell bodies in laminae I-III. Numerous neural processes were also observed in the spinal grey matter although there was little or no labelling of the white matter. Hybrid cells secreting antibody CE5 were recloned three times and all subsequent studies were performed on tissue culture supernatant. Antibody CE5 was found to be of the 1gM A subclass when tested by indirect immunofluorescence using subclass-specific antisera or after SDS-polyacrylamide gel electrophoresis of 35S-methionine-labelled immunoglobulin (gels run in reducing conditions with known 1gG and 1gM markers16).

Subpopulations of mammalian neurones stained by CE5

The distribution of cells labelled by CE5 was determined by visualizing antibody-binding sites with FITC- or peroxidase-conjugated rabbit anti-mouse IgM (as detailed in Fig. 1 legend).

In sections of adult rat dorsal root ganglion, the cytoplasm and external membrane of sensory neurones was labelled, whereas non-neuronal cells were not (Fig. 1a). A similar distribution and selectivity of labelling were seen in dorsal root ganglion cells grown in dissociated culture (Fig. 1b). Although insufficient CE5 was bound to the surface of viable neurones to enable detection by indirect immunofluorescence¹⁷, sufficient antibody was bound to activate complement and mediate lysis of >90% of sensory neurones. Significantly, non-neuronal cells in these cultures were unaffected by exposure to CE5 plus complement, consistent with the failure of CE5 to label non-neuronal cells by indirect immunofluorescence.

In 30-µm transverse sections of adult rat cerebellar cortex, Purkinje neurones were the only cells labelled (Fig. 2a) and showed intense staining of both the soma and dendritic tree, with occasional axonal labelling. Throughout the cerebral cortex, staining was observed in the cell bodies of pyramidalshaped neurones within layer V (Fig. 2d) and their basal and apical dendrites (Fig. 2e). The cell bodies of pyramidal-shaped neurones within layers II and III were faintly stained in some but not all cortical areas. Within the hippocampus, occasional pyramidal cell bodies and their dendrites were stained, as were the majority of granule cell bodies and mossy fibres in the dentate fascia. In the olfactory bulb, labelling was observed in mitral cell bodies and their dendrites projecting through the external plexiform layer and terminating in superficial glomeruli (fig. 2f), while the cell bodies of intrinsic olfactory neurones were unstained. A complex pattern of neuronal labelling with CE5 was observed in other regions of the central nervous system (to be described in detail elsewhere), although staining in all

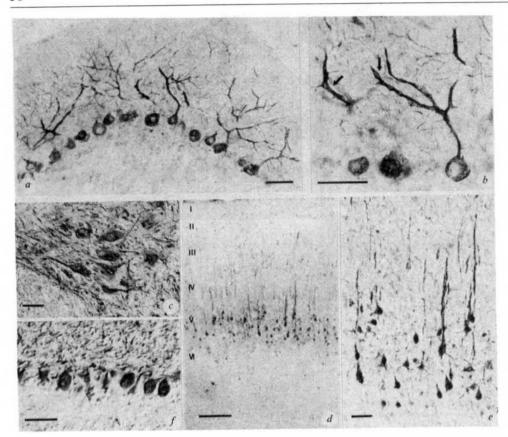


Fig. 2 Immunocytochemical localization of neurones in the rat central nervous system labelled with CE5. Immunoperoxidase staining procedures were carried out as described in Fig. 1 legend, a, Selective labelling of Purkinje neurone cell bodies and dendrites in rat cerebellar cortex. Scale bar, 100 µm. b, High power micrograph showing the cylindrical labelling pattern (arrows) in oblique sections through Purkinje neurone dendrites, suggesting an association of the antigen with the surface membrane of the dendrite. The Purkinje neurone cell body, however, is labelled throughout the cytoplasm. Labelled axons can occasionally be seen projecting through the granular layer. Scale bar, 100 µm. c, Staining of the cell bodies and initial processes motoneurones, and other neuronal elements within the ventral horn of the rat cervical spinal cord. Scale bar, 50 µm. d Selective labelling of neurones in or near layer V of the rat occipital cortex. Dendrites can be seen projecting to more superficial layers. Scale bar, 200 μm. e, High power micrograph showing the pyramidal appearance of labelled cell bodies within rat occipital cortex. Numerous superficial dendrites are stained and also occasional radial dendrites projecting within layer V Scale bar, 50 µm. f, Immunoperoxidase staining of mitral cell bodies and dendritic profiles in the olfactory bulb. Scale bar, 50 µm.

regions was confined mainly to neurones with axons projecting beyond their nucleus of origin.

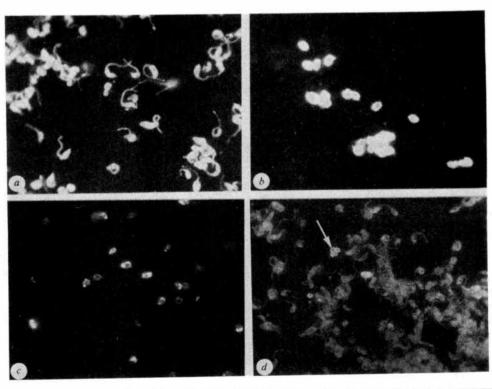
The specificity of neuronal labelling provides few clues, in itself, to the functional significance of CE5-defined antigens. Antigenic expression is clearly unrelated to the transmitter specificity of individual neurones and occurs in neurones derived from both the neural tube and neural crest. Other monoclonal antibodies recognizing subpopulations of vertebrate and invertebrate neurones reveal a completely different distribution of labelled cells¹⁷⁻²¹. In addition, although cerebellar Purkinje neurones and some but not all of the central

neurones stained by CE5 are also stained by polyclonal antisera directed against the calcium-binding proteins calmodulin²², parvalbumin²³ and vitamin D-dependent calcium-binding protein²⁴, CE5 did not react with any of these proteins. Antibody CE5 therefore seems to define a novel antigenic determinant in the mammalian nervous system.

Staining of trypanosomes by CE5

Of 11 monoclonal antibodies raised against rat neurofilaments, neuroglial cells or other neural antigens, only CE5 and its subclones stained formaldehyde-fixed *T.cruzi* when examined

Fig. 3 Staining of T.cruzi (Y Strain) organisms with CE5 monoclonal antibody. a, Formaldehyde-fixed epimastigotes showing homogeneous cytoplasmic fluorescence. b, Formaldehyde-fixed amastigotes showing bright fluorescence associated with the surface membrane. c, Viable amastigotes stained in suspension; living epimastigotes gave no reaction with CE5. d, Batch culture of epimastigotes containing a low percentage of amastigotes, after formaldehyde fixation. Epimastigotes gave typical cytoplasmic fluorescence whereas in amastigotes (arrowed) the antigen detected by CE5 is limited to the cell-surface membrane. As a specificity control, parasites were also treated with an IgM monoclonal antibody against dinitrophenol or with ascitic fluid containing MOPC 104E myeloma IgM. Positive staining was only observed with CE5.



by indirect immunofluorescence using FITC-conjugated antiimmunoglobulin serum. Epimastigotes of *T.cruzi* (Y strain) and *Herpetomonas samuelpessoai* were obtained from culture in Warren's medium²⁵. Non-motile amastigotes of *T.cruzi* were obtained from cultures of a murine thigh muscle tumour (S2) infected with trypomastigotes derived from the plasma of acutely infected mice. *T.brucei* trypomastigotes were isolated from the plasma of acutely infected rats. Whereas both epimastigotes and amastigotes of *T.cruzi* exhibited bright immunofluorescent staining (Fig. 3a, b), *T.brucei* and *H.samuelpessoai* showed no staining with CE5.

In addition, the various developmental stages of T.cruzi exhibit differential staining patterns with CE5. Paraformaldehyde-fixed epimastigotes reacted with CE5 to produce a homogeneous cytoplasmic staining (Fig. 3a) whereas staining of fixed amastigotes was only associated with the cell-surface membrane (Fig. 3b). The significance of this observation in relation to the manifestation of Chagasic symptoms would be greatly enhanced if this antigen was present on the surface of the parasite and thus directly available to the immune response. To examine this possibility, viable amastigotes and epimastigotes were incubated sequentially with CE5 and FITC-conjugated rabbit anti-mouse 1gM. The surface membrane of viable amastigotes exhibited a bright immunofluorescence (Fig. 3c) whereas no staining was observed on the surface of viable epimastigotes. In the conditions of batch cultures used in these experiments, some epimastigotes showed spontaneous transformation into amastigotes. These viable amastigotes also exhibited intense membrane staining (Fig. 3d).

CE5-defined antigens and Chagas' disease

In the peripheral nervous system, one of the main histological features associated with T. cruzi infection is periganglionitis and degeneration of intrinsic myenteric neurones in the gastrointestinal tract⁵. In fixed whole-mount preparations of guinea-pig submucosal plexus²⁶ treated with CE5, clusters of neuronal cell bodies exhibited bright immunofluorescence within the cytoplasm and external membrane (Fig. 4a). Staining of neuronal processes within the submucosal plexus was also observed. However, without prior removal of the extrinsic sensory nerves projecting to the gut, it is not possible to determine whether these fibres originate from enteric neurones or from vagal and spinal sensory neurones.

The acute and chronic phases of Chagas' disease are characterized by the degeneration of striated muscle fibres in the heart, whereas skeletal muscle does not appear to be affected²⁷. Staining of cardiac muscle fibres appeared as intense crossstriations (Fig. 4c). The spacing between stained bands was 2.2–2.4 μ m and sequential examination under bright field and phase contrast optics revealed staining located at the junction of A and I bands. The same pattern of staining was observed in glycerinated muscle fibres, suggesting that the antigen is closely linked to structural proteins in the muscle fibre. In contrast, CE5 did not stain skeletal muscle fibres removed from the rat tibialis anterior muscle.

The antigenic determinants recognized by CE5 are also present in the human nervous system; Purkinje neurones were stained selectively in postmortem human cerebellum (Fig. 4c). Although little is known about the central neuropathology in patients suffering from Chagas' disease, the only central neurones so far reported to undergo degeneration are cerebellar Purkinje neurones⁵.

Antigens recognized by CE5

The staining of Purkinje neurones in rat cerebellum could be abolished by preabsorbing CE5 hybridoma supernatant with an equal volume of formaldehyde-fixed *T.cruzi* epimastigotes, confirming that a common antigenic determinant is therefore expressed by mammalian neurones and *T.cruzi* parasites. If this determinant is relevant to the neuropathology associated with Chagas' disease, the serum of mammalian hosts infected

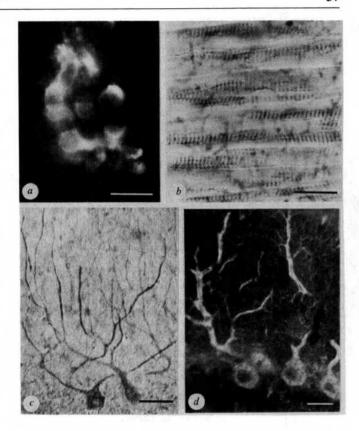


Fig. 4 CE5 labelling of mammalian cells known to degenerate in Chagas disease. a, Immunofluorescent labelling of neuronal cell bodies in ganglia located in the submucosal plexus of the guinea pig. Whole-mount preparations of the guinea pig submucosal nerve plexus²⁶ were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 30 min, washed and processed for indirect immunofluorescence as described in Fig. 1 legend. Scale bar, 50 µm. b, Immunoperoxidase labelling of cross-striations in cardiac muscle fibres in 30-um sections of formaldehyde-fixed rat heart exposed to CE5. Consistent staining of cross-striations can be observed. Apparent staining in the longitudinal axis reflects folds in the muscle fibre. Scale bar, 50 μm. c, Interference contrast micrograph showing immunoperoxidase staining of two Purkinje neurones in human cerebellar cortex. Human postmortem cerebellar cortex was obtained within 12 h of death and fixed by immersion for 24-48 h in 4% paraformaldehyde, washed for 24 h and processed for immunocytochemistry as described in Fig. 1 legend. Note the absence of staining of other neurones in the molecular layer. Scale bar, 100 µm. d, Immunofluorescent staining of rat cerebellar Purkinje neurones after exposure of cerebellar sections to sera obtained from mice infected 90 days previously with T.cruzi parasites. Sera from infected mice were diluted 1:10 with PBS and applied to cerebellar sections for 18-24 h at 4 °C. After extensive washing, sections were incubated with FITC-conjugated anti-mouse immunoglobulin (Miles) diluted 1:50 for 30 min at room temperature, washed and mounted on glass slides. Intense staining of the cell bodies and dendrites of the Purkinje neurones can be seen, with less intense staining of other cellular elements in the molecular layer. Scale bar, 50 µm.

with *T.cruzi* might be expected to contain circulating antibodies directed against the same determinant. Sera from mice infected with *T.cruzi* for 14, 90 or 210 days were therefore used to stain sections of rat cerebellum. An intense labelling of the cell bodies and dendrites of Purkinje cells was seen, with additional, weaker staining of other cells in the molecular and granular layers (Fig. 4d). Sera from uninfected mice gave no specific staining.

The determinants in mammalian cells and T.cruzi that are recognized by CE5 were analysed using the immune blotting technique of Towbin et al.²⁸. All rat neuronal tissue, of central or peripheral origin, produced a similar pattern (Fig. 5, lanes c, d). A predominant immunoreactive band with an apparent molecular weight (M_r) of $\sim 60,000$ was observed together with a minor band of $M_r \sim 32,000$ and very faint bands $M_r > 70,000$.

Extracts of cardiac muscle revealed an intense band of $M_r \sim 20,000$ (Fig. 5, lane e) whereas skeletal muscle showed no stained bands, in accord with the results found by immunocytochemistry. Several very faint bands were also

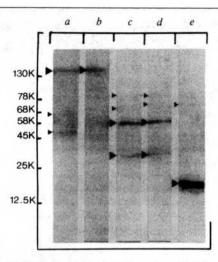


Fig. 5 Autoradiographic localization of proteins stained by CE5 after polyacrylamide gel electrophoresis and electrophoretic transfer of proteins onto nitrocellulose. Samples (50-100 µg) were boiled for 2 min in 10% SDS containing 2% \(\beta\)-mercaptoethanol and electrophoresed in 10% polyacrylamide gels in the presence of SDS. After electrophoresis, the gel was blotted onto a nitrocellulose sheet for 2 h at 12 V. The sheet was then incubated overnight in PBS containing 3% haemoglobin. CE5 tissue culture supernatant diluted 1:10 in PBS-haemoglobin was then applied to the sheet for 60 min. After six washes in PBS, ¹²⁵I-labelled rabbit anti-mouse globulins (5×106 c.p.m.) in PBS-haemoglobin was applied to the sheet for 60 min, and the sheet then washed a further six times in PBS. The dried sheet was exposed for 5-15 days at -70 °C using Kodak X-omat H film. Lane a, epimastigotes; lane b, amastigotes; lane c, 7-day rat dorsal root ganglia; lane d, rat cerebellum; lane e, rat heart. The positions of molecular weight standards are indicated.

observed in extracts of liver, kidney and testis and a very faint reaction was found in these immunochemistry. The staining patterns produced by extracts of T.cruzi epimastigotes and amastigotes were distinct, with bands in a molecular weight range of 50,000-100,000 (Fig. 5, lanes a, b). Extracts of T.brucei, however, exhibited no immune staining, again in agreement with immunofluorescence studies.

The complex banding pattern shown by CE5 may reflect a common amino acid sequence in distinct proteins, breakdown products of a single large protein or a carbohydrate moiety common to several glycoproteins. Exposure of rat cerebellar sections to neuraminidase, mannosidase or periodate oxidation at pH 7.4 (ref. 29) did not affect the staining of Purkinje neurones by CE5. Similarly, incubation in the presence of 100 mM α-methyl mannoside, sucrose, galactose or mannose did not reduce the binding of CE5 to Purkinje neurones. It is therefore probable that the CE5-defined antigen is a polypeptide structure present in several different proteins which share a common epitope. Such complexity in staining patterns obtained with monospecific antibodies is not unique. Monoclonal antibodies to glial fibrillary acidic protein³⁰, and to Thy 1.1 (ref. 31) have recently revealed unsuspected antigenic crossreactivity between distinct proteins.

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Conclusions

Although previous serological studies 10-13 have suggested that mammalian neurones, cardiac muscle and T.cruzi organisms might share common antigenic determinants, the identification of these antigens using monoclonal antibodies provides a more reproducible basis for future studies. At present, we have no direct evidence that the cross-reactive antigenic determinant recognized by CE5 is involved in the generation of Chagasic lesions. Previous experimental measurements of cell-mediated immunity against cardiac and neuronal antigens showed that a significant degree of autoimmunity was attained only after acute phase degeneration of cardiac neurones had occurred. The late appearance of anti-self-reactivity was taken as evidence that autoimmunity was the result of the pathogenic process and so could not be involved in its initiation. Although autoimmunity provoked by cross-reactive structures might still have a secondary role in the development of Chagasic lesions, the description of anti-neuronal autoantibodies soon after infection and coincident with neuronal destruction32 suggests that an antibody-mediated mechanism might initiate or amplify acuteand chronic-phase lesions in cardiac or neuronal cells.

The availability of CE5 antibody should allow a direct assessment of the role of cross-reactive antigens in the development of Chagasic lesions. For example, proteins expressing the antigen could be purified from mammalian cells or T.cruzi organisms using affinity columns and injected in mice. The appearance of Chagasic lesions would provide strong evidence for their involvement in the disease. It will also be important to determine the effect of central or peripheral administration of CE5 antibody to normal or T.cruzi-infected mice. In infected mice, it is difficult to predict the outcome of CE5 treatment: the result might be parasite and/or antigen clearance with consequent disease amelioration, or alternatively increased neuronal and cardiac damage with disease exacerbation.

The present report may have important implications for the clinical study of Chagas' disease. If the similarity in neuronal staining in rat and human brain extends beyond the cerebellum, it may be appropriate to examine the central neuropathy associated with Chagas' disease in more detail.

Only 1 of 11 antibodies raised against mammalian neural antigens has revealed a cross-reactivity with T.cruzi. further studies using monoclonal antibodies directed against peripheral and central neurones will reveal whether other shared determinants exist and may also provide a greater variety of immunological probes with which to study Chagas' disease.

This work was supported by grants from the Royal Society, the Wellcome Trust and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. T.N.W. and T.M.J. were supported by an MRC project grant and a Royal Society Locke Research Fellowship respectively. We thank our colleagues at St George's Hospital Medical School for help during these experiments, Dr B. H. Anderton and Professor J. S. Kelly for constructive comments, Professor M. Matsushita and Dr T. Endo for anatomical advice and Dr M. Noble for advice on cytotoxicity experiments. P. Hamilton and S. J. Morgan provided technical assistance.

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Regulation of metallothionein-thymidine kinase fusion plasmids injected into mouse eggs

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A plasmid was constructed with the promoter/regulatory region of the mouse metallothionein-I gene fused to the structural gene of herpesvirus thymidine kinase. When mouse eggs were microinjected with this plasmid and incubated with cadmium (a natural inducer of metallothionein gene transcription) thymidine kinase activity increased ~ 10 -fold compared with control eggs not exposed to cadmium. Analysis of a set of deletion mutants revealed that the minimum sequence required for cadmium regulation lies within 90 nucleotides of the transcription start site.

METALLOTHIONEINS (MTs) are ubiquitous proteins of vertebrates and invertebrates that bind heavy metals and are thought to be involved in zinc homeostasis and resistance to heavy metal toxicity¹. The metallothionein-I (MT-I) gene is controlled at the transcriptional level by heavy metals and by glucocorticoid hormones²⁻⁴. To ascertain which sequences are involved in transcriptional regulation, an assay system is required which allows faithful transcription and responds to regulatory molecules. There are currently three basic approaches: cell transformation⁵⁻⁹, cell-free transcription^{10,11} and microinjection of DNA into cells. Plasmids containing intact eukaryotic genes or various mutations created from them in vitro have been injected into the germinal vesicle of frog oocytes to assess the nucleotide sequences required for synthesis of functional mRNAs encoding histones¹², globin¹³, ovalbumin¹ and thymidine kinase¹⁵. Mouse oocytes have been used to study the expression of injected 5S ribosomal genes¹⁶ and mammalian tissue culture cells have been transformed by microinjection of DNA^{17,18}. DNA injected into fertilized mouse eggs may be retained in the adults that develop after reimplanting the eggs into foster mice and in some cases the foreign DNA is expressed 19-23. However, use of mammalian cells for quantitative analysis of transient expression of microinjected plasmids has not previously been reported.

Here we establish the usefulness of microinjecting plasmids into mouse ova as a convenient system for studying transcriptional regulation. The major advantages of this system are that it is homologous to the promoter under study, which should optimize the chances for faithful transcription and regulation, and it is rapid compared with cell transformation. To distinguish transcription originating from the injected genes from that of the endogenous genes, we have fused the mouse MT-I gene promoter and 5'-flanking region to the thymidine kinase (TK) gene of herpes simplex virus (HSV). Viral TK is readily assayed and can be distinguished from endogenous TK^{24,25}. Our results indicate that the MT-I promoter in the fusion plasmid is as effective as the normal HSV TK promoter in allowing synthesis of functional TK when injected into fertilized mouse ova. Furthermore, the MT-I promoter and adjacent DNA sequences respond to Cd and regulate the production of viral TK.

Expression of viral thymidine kinase in mouse ova

In the first experiments, TK activity was monitored by measuring the incorporation of ¹²⁵I-deoxycytidine (¹²⁵I-dC) into DNA. This assay exploits the fact that the HSV TK can phosphorylate ¹²⁵I-dC whereas the mammalian enzyme cannot²⁴; hence, the

Table 1 HSV TK activity in mouse fertilized one-cell eggs injected with various plasmids

Plasmids injected	Molecules of plasmid injected per egg	Incorporation of ¹²⁵ I-dC into DNA* (c.p m.)	Formation of ³ H-TMP† (c p.m.×10 ⁻³)
2			
None	******	99 ± 18	
pBR322	4,400	101 ± 13	
pMT	2,300	80 ± 10	
pTK	2,300	$1,230 \pm 310$	
pMT-TK	1,600	$2,860 \pm 940$	
pMK	2,000	$2,900 \pm 480$	
pMK	200	470 ± 30	
pMK	20	180 ± 50	
b			
None			9.06 ± 0.64
pBR322	2,000		7.35 ± 0.17
-	20,000		9.17 ± 1.1
pTK	2,000		94 ± 22
•	20,000		870 ± 205
pMK	2,000		170 ± 21
	20,000		970 ± 155

Fertilized one-cell ova were flushed from the oviduct using Brinster's medium. The morning of day 1 of pregnancy. Cumulus cells were removed from ova with hyaluronidase (300 U mi⁻¹), and the ova were washed free of debris and enzyme before manipulation. For injection, the ova were transferred to a depression slide in Brinster's medium containing 5 µg mi⁻¹ cytochalasin B and held in place using a blunt pipette while the tip of the injector pipette was inserted through the zona pellucida and vitellus and into the cell nucleus. The DNA solution in the injector pipette was slowly discharged into the nucleus using a syringe connected to a micrometer. The larger pronucleus (male) of the fertilized ovum was injected with ~2 pl of plasmid solution. The injection volume was estimated from the increase in diameter of the nucleus. After injection the ova were washed free of cytochalasin and returned to the same medium used for collection. When the injections were completed the ova were incubated for 22 h in 25 µl of medium. The plasmids injected were: pBR322, the vector plasmid into which the genes were cloned; pMT, which contains the mouse MT-12 gene; pTK, which contains HSV TK27; pMT-TK, which contains both the MT-I and HSV TK structural gene (Fig. 1).

contains the promoter of MT-I fused to the HSV TK structural gene (Fig. 1).

* Values are c p.m.+s.e.m of ¹²⁵I-dC (0 5 µCi; >700 Ci mmoi⁻¹; NEN) incorporated into DNA by 25 ova during a 22 h incubation period

† TK assays were performed in the following manner. After injection of the plasmid, the ova were incubated for 22 h in Brinster's medium then transferred to 20 μ l of hypotonic buffer (10 mM KCl, 2 mM MgCl₂, 10 mM Tris-HCl, pH 7.4, 1 mM ATP, 10 mM β -mercaptoethanol, 50 mM ϵ -aminocaproic acid and 1 mg ml⁻¹ bovine serum albumin)²⁹. The cells were frozen and thawed three times and 20 μ l of reaction mixture (150 mM Tris-HCl, pH 7.5, 10 mM ATP, 10 mM MgCl₂, 25 mM NaF, 10 mM β -mercaptoethanol) were added followed by 5 μ l of water containing 5 μ Ci ³H-thymidine (80 Ci mmol⁻¹; NEN). The mixture was incubated for 2 h at 37 °C, and the ³H-TMP produced was measured by adsorption on to DE-81 paper and subsequent scintillation counting ^{30.31} The values are c.p.m. + s.e.m. of ³H-TMP formed by 25 ova injected with the indicated number of plasmids and incubated for 22 h before assay.

background is very low. Table 1 compares TK activity measured after injecting a few thousand copies of plasmids pBR322, pMT (which contains a 3.8 kilobase (kb) MT-I gene fragment²⁶ inserted into the EcoRI site of pBR322), pTK (which contains a 3.5-kb HSV TK fragment²⁷ inserted into the BamHI site of pBR322), pMT-TK (which contains both MT-I and TK genes²³) and pMK (which has the MT-I gene promoter and 5'-flanking region fused to the TK gene²³; the predicted DNA sequence around the fusion site is shown in Fig. 1). Table 1a shows that incorporation of 125 I-dC into DNA during a 22 h incubation was the same as background when pBR322 or pMT were injected, but increased at least 10-fold when plasmids containing an intact TK gene were injected. A similar high level of 125 I-dC incorporation was observed when pMK was injected. Significant incorporation of ¹²⁵I-dC was detected with as few as 20 molecules of pMK injected per ovum. Table 1a indicates, however, that incorporation of ¹²⁵I-dC was not linearly related to plasmid copy number injected; in other experiments, injection of 20,000

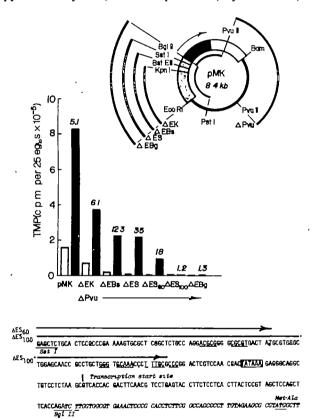


Fig. 1 TK activity generated from various 5'-deletion mutants of pMK. The plasmid is shown at the top. It was generated by restriction pMT-TK with genes, followed by ligation of the MT-I 5' sequences to the TK structural gene²³ The mouse DNA sequence of pMK is referred. BglII, which cuts just upstream of the initiation codon of both MT-I and TK gene²³ The mouse DNA sequence of pMK is stippled, the HSV TK structural gene solid, the 3' HSV sequences are open, and the pBR322 sequences are shown as a solid line. The arrow indicates the direction of transcription. The Puull fragment of pMK was removed as shown by ΔPvu, then a series of deletions were made that extend from EcoRI toward the 5 end of the TK gene. The Ssfi, BstEII and KprI sites he ~150, 340 and 600 bp 5' of the transcription start site, respectively Bal31 was used to extend deletions from Sril site towards the TK gene. In each case an EcoRI linker was inserted and then ligated to the original EcoRI site to create deletions that extend from EcoRI to the positions indicated by the arrows (±5 bp) above the DNA sequence shown at the bottom; the end points were mapped by acrylamide gel electrophoresis of RsaI/EcoRI digests. The MT-I sequence³⁵ between SsI and BgIII is shown fused to the HSV TK sequence³⁴ at the BgIII site. Two palindromes, the TATAAA box, the transcription start site and the initiation codon for TK are indicated Approximately 2,500 molecules of each plasmid were microinjected; TK activity was measured 22 h later as described in Table 1b legend. The results are the means of two experiments with each deletion mutant. Eggs were incubated with (solid histograms) or without Cd (open histograms) for 22 h before TK assay. The numbers over the histograms indicate the ratio of TK activity with and without Cd. The background TK activity of the eggs (2,000-5,000 c.p.m.) has not been subtracted The activities of mutants ΔES_{100} and ΔEBg are not significantly different from background.

or 200,000 copies of pMK gave no more incorporation than injection of 2,000 copies. We suspect that these results reflect the limited capacity of the fertilized ovum to synthesize DNA; hence, when large amounts of plasmids are injected, DNA synthesis becomes rate limiting rather than conversion of I-dC to I-dCMP by viral TK.

To obviate this problem, we determined TK activity directly by measuring the conversion of ³H-labelled thymidine to ³H-TMP by homogenates of ova prepared 22 h after plasmid injection. With this assay, enzyme activity was nearly linear when up to 20,000 copies of plasmid were injected per ovum (Table 1b). Thus, the indirect assay with ¹²⁵I-dC provides sensitivity to small numbers of injected genes while the direct assay with ³H-thymidine is more useful when large numbers of genes are injected.

The results with pMK suggest that the MT-I gene promoter is substituting for the TK gene promoter to allow transcription of a functional TK mRNA. To ascertain whether the 5'-flanking region of the MT-I gene also allows regulation of transcription by heavy metals or glucocorticoids, the injected ova were incubated for 22 h with or without 50 μM Cd or 100 nM dexamethasone before TK assay. Table 2 shows that Cd treatment results in a dramatic increase in TK activity when 200 or 2,000 gene copies were injected per ovum. When 20 gene copies were injected the response to Cd was low; however, when the more sensitive ¹²⁵I-dĈ technique was used, a 2.5-fold increase in TK activity was detected after Cd treatment (data not shown). The amount of TK activity obtained when plasmid pTK was injected was unaffected by Cd treatment (data not shown). These results indicate that the molecules responsible for heavy metal regulation of MT-I gene transcription are present in mouse ova and that the regulatory sequences are contained within the 1.7 kb of mouse DNA 5' of the BglII site. We have not detected any increase in TK activity in response to dexamethasone treatment using either assay. This may be due to the absence of functional glucocorticoid receptors, lack of necessary DNA sequences, or inappropriate chromatin structure.

We attempted to eliminate gene activity by cutting the pMK plasmid between the MT-I promoter and the TK structural gene at the BglII site (see Fig. 1). Some of the linearized molecules were treated with DNA polymerase I (Klenow fragment) to fill in the 'sticky ends' generated by BglII. In both cases the linearized molecules were separated from any uncut molecules or nicked circles by agarose gel electrophoresis. Table 3 shows that despite the separation of MT-I promoter from the TK structural gene, the injected ova still produced a high level of TK activity, whether or not the DNA ends were blunted with DNA polymerase I. Furthermore, the TK activity was greatly enhanced by Cd treatment. A likely explanation for these results is that the mouse ovum has the requisite enzymes necessary to re-ligate the linearized pMK plasmid. Comparison of the TK activity generated with different dosages of plasmids indicates that the activity obtained with the blunt-ended fragment is essentially the same as with supercoiled plasmid (compare Tables 2 and 3), suggesting that re-ligation is very efficient. Analysis of DNA injected into eggs by agarose gel electrophoresis and Southern blotting confirms that linear DNA molecules become re-ligated and supercoiled (data not shown).

To examine this phenomenon further, the pMK plasmid was cut to yield individual 'regulatory region' (PsI to BglII) and 'structural gene' (BglII to PvuII) fragments (see Fig. 1, inner circle). Each fragment was injected separately and in combination, as shown in Table 3. When high levels (4,000 per nucleus) of the separate fragments were injected no significant increase in TK activity above control levels was observed. Furthermore, injection of 4,000 molecules of both fragments did not result in demonstrable TK activity. However, re-ligation of the fragments in vitro followed by injection resulted in high levels of TK activity which were further elevated by Cd treatment. Injection of higher levels (24,000 molecules) of the separate fragments was also effective. Our interpretation of these results is that the

individual 'regulatory region' and 'structural gene' sequences do not have TK activity and that correct ligation of the separate fragments is inefficient compared with the circularization of the linearized plasmid.

Deletion mutants define promoter and regulatory sites

To define more precisely the mouse DNA sequences required for cadmium regulation we prepared a set of deletion mutants. The first step entailed deletion of a 3.0-kb PvuII fragment including the 3'-flanking region of the TK gene and nonessential pBR322 sequences; this fragment includes an EcoRI site that complicates construction of subsequent deletions. A set of deletions extending from the remaining EcoRI site towards the TK gene was then prepared by restriction with specific enzymes and re-ligation of blunted ends, as indicated in Fig. 1. To extend further towards the TK gene from the SstI site, the double-strand exonuclease Bal31, was used and an EcoRI linker inserted to facilitate mapping. The approximate (± 5 base pairs [bp]) end points of these deletions are indicated by arrows on the sequence at the bottom of Fig. 1. Figure 1 (histograms) shows that there was a progressive loss of constitutive as well as Cd-induced activity as the 5' sequences were deleted. TK activity measured in the absence of Cd was more severely decreased than in its presence; thus the ratio of activity with and without Cd increased (as indicated by the numbers over the histograms) until the minimal sequence required for Cd regulation was reached. The minimal sequence tested that preserves Cd regulation (\Delta ES_{60}) retains ~90 nucleotides of mouse DNA 5' of the transcription start site and includes a large palindrome centred at position -55. Deletions that extend into this palindrome (ΔES_{100}) or delete all the mouse sequences (ΔEBg) have no activity (<1.5 times background) and are not inducible by Cd. These data indicate that the 5' MT-I sequence carries both promoter and Cd regulatory sites. Although a minimal sequence can be described (exemplified by ΔES_{60}), additional sequences that potentiate both basal and induced activities clearly extend at least to the KpnI site, which is ~ 600 bp 5' of the transcription start site.

We conclude that fusion of the MT-I and HSV TK genes at their respective BglII sites results in a gene that is transcribed, presumably by RNA polymerase II4, and that the mRNA is translated into functional TK. Furthermore, the gene fusion is regulated by Cd in a manner similar to the endogenous MT-I gene. We have as yet been unable to dissociate a functional promoter from cadmium regulation. By 5'-deletion analysis we have identified a minimal control region that includes ~90 nucleotides 5' of the transcription start site. Plasmids that contain only 50 nucleotides 5' of the transcription start site are inactive plus or minus cadmium, despite the fact that they retain the TATAAA sequence that is generally required for transcription in vitro. These boundaries lie close to those defined by

Table 2 Cd regulation of the amount of TK activity produced from pMK

	Formation of ³ H-TM	MP (c.p.m.×10 ⁻³)*
No. of pMK plasmids injected	Ova cultured without Cd	Ova cultured in 50 µM Cd
2,000	190.0	2,400
200	25.3	270
20	8.3	12.2
0	8.5	8.8

Ova were injected with the number of pMK plasmids indicated as described in Table 1 legend. The ova were then randomly divided into two groups and incubated for 22 h with either 0 or 50 µM Cd in the medium. TK activity was measured as described in Table 1 legend.

* Each value is the mean of ³H-TMP formed by 25 ova in two separate experiments.

Table 3 TK activity of restriction fragments derived from pMK

Plasmid treatment*	No. of DNA molecules injected		n of ³ H-TMP .×10 ⁻³)† +50 μM Cd
PMK restricted with BglII	2,000	92.6	1,000
pMK restricted with Bg/II	2,000	68.3	1,480
and blunt-ended with DNA	400	50 8	514
polymerase I	80	5.1	39.9
TK 'structural gene' fragment (BglII to PvuII)	4,000	6.8	6.5
MT-I 'regulatory region' fragment (PxI to Bg/II)	4,000	7 8	11.4
TK 'structural gene' and MT-I 'regulatory region' re-ligated in vitro	4,000 of each	105	407
TK 'structural gene' and MT-I	4,000 of each	11.9	10.1
'regulatory region' injected as separate fragments	24,000 of each	139	595
No plasmid injected	0	8.4	7.9

* Plasmid pMK was restricted with Bg/II; one-half of the sample was incubated with DNA polymerase I (Klenow fragment) in the presence of all four deoxyribonucleotide triphosphates. Then the DNA was electrophoresed on a 0.7% agarose gel; the linear band was localized by ethidium bromide staining (no supercoils or nicked circles were visible) and the DNA was eluted from the gel by the NaClO₄ method³² The TK 'structural gene' and MT-I 'regulatory region' fragments were stolated by cutting pMK with PstI, Bg/II and PvuII and separating the fragments on a 1% agarose gel and eluting them as described above. For re-ligation in vitro, 1.2 µg of the PstI-BglII fragment (2,500 bp) was mixed with 0 9 μg of the Bg/II-PvuII fragment (1,700 bp) in a 50 μl reaction mixture with T4 ligase and incubated for 6 h at 15 °C After ligation the mixture was extracted with SDS, phenol and chloroform, and the DNA was precipitated with ethanol.

† Ova were injected with the indicated number of DNA molecules and incubated for 22 h with (+) or without (-) 50 μ M Cd before TK assay as described

in Table 1 legend.

McKnight et al. 15 for the TK promoter. However, in contrast to their results, we find that sequences further upstream of -90 potentiate transcription. Some of these sequences allow transcription in the absence of Cd and may, therefore, represent additional promoters. Similar potentiating effects of upstream DNA sequences have been described for histone genes¹². The nature of the molecules involved in heavy metal regulation of MT-I genes is unknown, but they are apparently ubiquitous because we have demonstrated heavy metal regulation of MT-I genes in a wide variety of mouse organs², cell culture lines³ and now mouse oocytes and eggs. This feature may be useful for constructing expression vectors that can function in a wide variety of cell types. Another possibility we are exploring is that this regulatable gene can be introduced into mice by injecting plasmids into fertilized eggs and then implanting the eggs into pseudopregnant mice. The basic feasibility of this approach has been demonstrated²³ and preliminary results indicate that this fusion gene is indeed regulated in mouse liver.

We thank Myrna Trumbauer for assistance, Steven McKnight for sending us a preprint of his manuscript, and S. Kit and A. Senear for helpful comments and suggestions. Financial support was from NIH grant HD 12384 and NSF grant PCM 78-22931 to R.L.B. and NIH grant HD 09172 to R.D.P. H.Y.C. was a trainee on HD 00239.

Received 12 May, accepted 21 December 1981

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Association of PSR0740-28 with an H I shell in Puppis

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During a survey of the interstellar atomic hydrogen (H I) distribution in the Puppis region of the Milky Way, we detected numerous, sometimes expanding, HI shells and filaments, indicative of past energetic explosive events in this area of the Galaxy. One of these shells, GS241-01+15, appears to be physically associated with the young pulsar PSR0740-28. In particular, the pulsar is coincident in position with an HI hotspot on the rim of the shell, and there are two nearby non-thermal radio sources, one of which lies on the rim of the H I shell coincident with the position of the open cluster Hf11, the other lying near the centre of the shell. Both non-thermal sources have been previously identified as possible supernova remnants (SNRs)^{1,1}. Furthermore, velocity measurements of the peak emission from the hydrogen shell and of the 21-cm absorption spectrum of the pulsar offer strong confirmation that the pulsar and the HI shell lie at the same distance from the Sun. This result is of interest because very few pulsars have been linked to known supernova remnants, and only the Crab and Vela pulsars with any degree of certainty3. Whether the pulsar PSR0740-28 is the remnant of the supernova progenitor of the HI ring, or perhaps the remnant of an early supernova in a young stellar group which was formed by the expanding HI shell, is as yet unclear. Present data seem to support the latter interpretation, suggesting that PSR0740-28 may have resulted from a 'second-generation' supernova event.

The correlation between pulsar and shell positions was noted during an analysis of a 21-cm neutral hydrogen survey of a region of the galactic disk known as the Puppis window. This unique area of the Milky Way contains much less absorbing interstellar dust and associated gas than is found in more typical regions of the Galaxy. In fact, galaxies have been observed near the galactic equator^{4,5}. It is estimated that the total visual extinction out to the edge of the galactic disk in the direction of galactic longitude $l = 245^{\circ}$ is only four to five magnitudes⁴. This is a favourable longitude for study of large-scale galactic kinematics as it is near the middle of the third quadrant of galactic longitude where circular galactic rotation provides a near optimum ratio of radial velocity to distance. Hence, this region of the Galaxy is ideally suited for the comparison of optical and radio observations, extending out to much greater

distances than is normally feasible in other portions of the galactic disk. In addition, large holes and gaps in the HI gas distribution, in velocity space, make this region particularly suitable for the identification of shells and ring-like structures, as a lack of foreground and background gas along various lines-of-sight minimizes the confusion of sources which would otherwise result.

The radio observations were conducted on the 43-m National Radio Astronomy Observatory (NRAO) radio telescope at Green Bank, West Virginia during two observing sessions: 22-28 June 1979 and 12-19 May 1980. The area observed consists of a 61×61 grid of points in the sky, extending from galactic longitudes $l = 239^{\circ}$ to 251° and from galactic latitudes $b = -9^{\circ}$ to $+3^{\circ}$, every 0.2° in l and b. The half-power beamwidth of the telescope at the 21-cm wavelength corresponding to neutral hydrogen emission is 21 arc min. The main results of our survey, along with a detailed description of our data reduction procedure, will be presented elsewhere⁶.

The H I shell which we denote by GS241-01+15 (GS refers to galactic shell, followed by position and velocity information in the format $l + b + v_{LSR}$, where all velocities have been referred to the local standard of rest (LSR)) was previously noted by Heiles⁷ in his analysis of the Berkeley low-latitude survey of neutral hydrogen⁸, and labelled as GS242-01+11. The finer spatial and velocity resolution of the present survey accounts for the differences in position and velocity range reported for this object. The centre of this HI shell coincides with an extended (1.2°×1.2°) radio source G240.8-1.2 discovered in a 29.9-MHz continuum survey by Jones and Finlay, and tentatively identified as a SNR1. The more recent 408 MHz maps of Haslam et al.9 contain a hint of this ring-like HI feature, though the correlation is far from exact.

The pulsar position ($l = 243.8^{\circ}$, $b = -2.4^{\circ}$) places it on the eastern edge of the HI shell, coincident with a fairly strong peak of 21-cm emission (HI cloud GC244-03+15) and just adjacent to another extended $(1^{\circ} \times 1^{\circ})$ non-thermal radio source G242.5-3.5 (see Figs 1 and 2)¹. Gomez-Gonzalez *et al.*¹⁰ detected strong absorption towards the pulsar in their observations with the Nançay radio telescope, having a velocity resolution of 12.7 km s⁻¹. Half of the pulsar signal is absorbed in their channel centred at +19.5 km s⁻¹, indicating that the absorption extends to at least the centre velocity. No absorption at all is seen in the next channel, centred at +32.2 km s⁻¹, indicating that the absorption has stopped before the lower velocity range of this channel, at ~+26 km s⁻¹. Thus absorption ceases between +19.5 and +26 km s⁻¹. The highest velocity at which we can still recognize the shell GS241-01+15 in emission is at about $+20~\rm km~s^{-1}$ where it blends in with strong generalized emission. This general emission peaks near $+20 \,\mathrm{km} \,\mathrm{s}^{-1}$ but extends to about $+30 \,\mathrm{km} \,\mathrm{s}^{-1}$ (see ref. 8). Since HI clouds which absorb the pulsar's signals must lie closer to us than the pulsar and HI clouds which do not appear in absorption presumably lie beyond the pulsar, the observed absorption indicates that the pulsar is at least as distant as the shell, and yet is not as distant as the numerous clouds causing the widespread HI emission at slightly higher velocities (and which are presumably lying just beyond the shell). As discussed

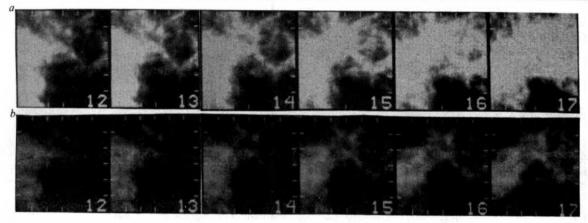


Fig. 1 Synthetic photographs of the constant-velocity neutral hydrogen (H I) distribution in the vicinity of the H I shell GS241-01+15 over the LSR velocity range +12 to +17 km s⁻¹. Tick marks are included in each map at 2° intervals at odd values of galactic longitude and latitude. Each map extends from $l = 239^{\circ}$ to 251° (right-to-left) and from $b = +3^{\circ}$ to -9° (top-to-bottom). a, Highlights H I brightness temperatures over the range 5-50 K; b, over the range 25-105 K. The pulsar PSR0740-28 lies on the hotspot ($l = 243.6^{\circ}$, $b = -2.6^{\circ}$) on the lower left rim of the shell.

by Weisberg et al.¹¹, bright H I emission clouds in the Milky Way rarely fail to provide significant absorption against extragalactic radio sources. Thus the pulsar PSR0740-28 lies not only on a hotspot of the H I shell in position, but also coincides with it in distance. The most recent distance estimate to this object, derived from a combination of dispersion measurements and H I absorption observations, is listed as 1.5 kpc (ref. 12), very nearly equivalent to our estimated kinematic distance of 1.4 kpc to the H I shell. We now consider possible physical mechanisms connecting the pulsar and the shell.

A summary of the observed and derived parameters describing the pulsar PSR0740-28, the H I shell GS241-01+15 and the H I cloud GC244-03+15 is given in Table 1.

PSR0740 – 28 is a very short-period pulsar (currently eighth-ranking of 330 pulsars by pulse period), whose measured period derivative implies a characteristic age of 1.58×10^5 yr (refs 12, 13). Striking similarities between PSR0740 – 28 and the Vela pulsar in terms of pulse shape and polarization properties have been noted ¹⁴. At the time of its discovery ¹⁵, it was noted that the pulsar PSR0740 – 28 was among the youngest detected without apparent association to a known SNR ¹⁵.

To determine more precisely the relationship of the H I shell GS241-01+15 to the pulsar PSR0740-28, it is of interest to estimate the age of this presumed SNR for comparison. Unfortunately, most models $^{16.17}$ relating the linear diameter of a SNR to its age are of questionable applicability in the present case as their results are appropriate for relatively young SNRs in the Sedov adiabatic expansion phase of their development. Our shell GS241-01+15, however, exhibits no appreciable variation in angular size with velocity, implying minimal expansion. This fact, coupled with the large linear size of the shell (~ 170 pc), leads us to believe that, if anything, GS241-01+15 is in transition from the isothermal expansion phase of its existence to the extinction phase, when the SNR begins to lose its identity as it merges with the interstellar medium.

Substituting typical values for initial supernova energy and ambient interstellar density into equation (1) of Herbst and Assousa¹⁸ yields an age in the range 10⁶-10⁷ yr for the shell GS241-01+15. Although the assumption of an unusually large supernova energy might bring the pulsar and shell ages into closer agreement, the indication is that such a large diameter H I shell has an age greater than that of PSR0740-28. Nonetheless, even disregarding the large uncertainties inherent in these estimates, such discrepancies between pulsar and SNR ages in reported associations (up to two orders of magnitude in some cases^{2,19}) have been said to have important implications in such fundamental areas as "supernova collapse theory, pulsar

acceleration mechanisms, and the birth rate and evolution of the galactic pulsar population".19.

Another factor to be considered is the displacement of the pulsar from the centre of the H I shell. Taking an angular separation between the pulsar and shell centre of $\sim 3.1^{\circ}$, and a distance of ~ 1.4 kpc, one finds that the pulsar is linearly displaced by about 75 pc from the centre of the H I ring. Assuming that the pulsar was originally at the centre of the H I shell, one may calculate its average annual proper motion, over a presumed lifetime of 1.58×10^{5} yr. This value is found to be ~ 70 m arc s yr⁻¹, corresponding to a space velocity of ~ 500 km s⁻¹. Though large (even for such notorious high-velocity objects as pulsars), at least two pulsars have been

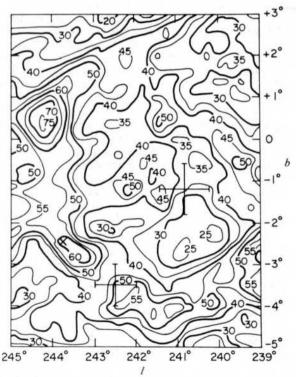


Fig. 2 Contours of 21-cm brightness temperature at an LSR velocity of $+15 \text{ km s}^{-1}$ in the vicinity of the H I shell GS241 -01+15. ×, The position of the pulsar PSR0740 -28 at $(l=243.8^{\circ}, b=-2.4^{\circ}).$ +, The positions of the non-thermal radio sources G240.8 -1.2 and G242.5 -3.5; the length of the bars indicates the approximate angular extent of these features (from ref. 1).

Table 1 Observed and derived parameters describing GS241-01+15, GC244-03+15 and PSR0740-28

	l_{ctr}	$b_{ m ctr}$ (de	Δ <i>l</i> g)	Δb	V _{mun} (kr	$\sum_{ ext{max}}^{ ext{V}_{ ext{max}}} V_{ ext{max}}$	Δr (deg)	d (kpc)	<i>LD</i> (pc)	$N_{ m H~I} \over ({ m cm}^{-2})$	n (cm ⁻³)	<i>M</i> (<i>M</i> _o)
Shell Cloud	241. 243.6	−1. −2.6	6. 0.75	8. 0.75	+7 +7	+20 +20	1.75	1.4 1.4	170 18	$\begin{array}{c} 2.4 \times 10^{20} \\ 5.0 \times 10^{20} \end{array}$	0.7 10	4.6×10^4 9.4×10^2
Pulsar PSR	0740-28	<i>l</i> 243.8	(deg) -2.		P s) 668	<i>p</i> (10 ⁻¹⁵) 16.832	H1ab (km: 0 to +	s ⁻¹)	d (kpc) 1.5	Age (yr) 1.58×10 ⁵		

Column headings for the HI shell and cloud are: lctr, bctr, galactic longitude and latitude of the centre of the radio features; Δl , Δb , approximate angular extent of the radio features in l and b (estimated to the nearest quarter degree); V_{min} , V_{max} , minimum and maximum LSR radial velocities between which the HI features are detected; Δr , the angular radial thickness of the HI shell (estimated to the nearest quarter degree); d, distance to the features from the Sun, calculated by applying a flat galactic rotation curve and using the LSR velocity of peak emission; LD, linear diameter of the H I features; $N_{\rm H I}$, H I column density within the shell or cloud (cm⁻²); n, particle number density within the shell or cloud (cm⁻²), assuming 10% helium abundance by number; M, total mass contained within the shell or cloud expressed in solar masses, assuming 10% helium abundance by number. Pulsar column headings: l, b, galactic longitude and latitude; P, pulse period; P, period derivative; HI absorp., LSR velocity of HI absorption; d, distance from the Sun; Age, characteristic age. All pulsar parameters are from ref. 12 except column 5 which is from ref. 10.

observed with transverse velocities in excess of 500 km s⁻¹ (refs 20, 21), and pulsar velocities up to several thousand km s⁻¹ have been repeatedly invoked to explain previously reported pulsar-SNR associations^{2,19}.

An alternate hypothesis regarding the origin of the pulsar is that the supernova which gave birth to PSR0740-28 was not the same one as that which gave rise to the HI shell. In other words, the pulsar has resulted from a relatively recent supernova event in a very young stellar group which was formed by the gradual expansion of the much older H i shell into an adjacent HI cloud, with subsequent shock-wave induced star formation. The presence of the pulsar in a hot spot on the HI shell, just adjacent to a non-thermal radio source, supports this interpretation. No obvious evidence of this second later supernova should be expected in our current HI observations, as the expected size of such a distant and young H I shell would be comparable to the beam size of the 43-m NRAO antenna. Such a second hypothesis supports the results of Morris et al.2 in naming the non-thermal source G242.5-3.5 as the likely point-of-origin of PSR0740-28, while simultaneously explaining its proximity to the non-thermal source G240.8-1.2 and the extended H I shell GS241-01+15, remnants of a much earlier supernova.

The position angle of PSR0740 -28 relative to G242.5 -3.5is 110°, and relative to G240.8-1.2 is 173°. According to Morris et al.², the intrinsic polarization vector for PSR0740 -28lies at 108°±4°, and the former source is the preferred point of origin for the pulsar, as it places the quantity (polarization angle minus position vector) close to the secondary peak (at 0°) in the probability distribution of this quantity as plotted for several pulsars. The angular separation between PSR0740-28 and G242.5 – 3.5 (\sim 1.7°) also implies a more reasonable transverse velocity of \sim 250 km s⁻¹. A logical follow-up to this hypothesis would be proper motion studies of PSR0740-28. The proper motions mentioned above should be detectable, and offer the means of differentiating between the H I shell and G242.5-3.5 as the more likely origin of the pulsar. Note how cloud GC244-03+15 is apparently elongated along the line joining the pulsar to G242.5-3.5. The position of G242.5-3.5also falls within the boundaries of the compact open cluster OCl-657 = Hf11 $(l = 242.41^{\circ}, b = -3.56^{\circ})^{22}$. Other open clusters which have been associated with SNRs are Tr18 (ref. 23) and NGC559 (ref. 24). Very little is currently known about Hf11, which is also listed as cluster number 3 in the compilation of van den Bergh and Hagen²⁵, and thus we cannot determine whether its age is consistent with the age of the HI shell. Clearly, a programme of photometry and spectroscopy on this cluster is necessary to determine its age.

Other clusters in the general vicinity which may be related to the H I shell GS241 – 01 + 15 are Tr9 ($l = 243.1^{\circ}$, $b = +1.2^{\circ}$) and NGC2453 ($l = 243.3^{\circ}$, $b = -0.9^{\circ}$). Fenkart and Binggeli²⁶ give distances and earliest spectral types of 2.23 kpc and b5 for

Tr9, and 1.49 kpc and b7 for NGC2453. A search for very young stars and molecular clouds in the vicinity of the pulsar and near other H I hotspots on the shell might provide further evidence for possible shock-wave induced star formation along the eastern rim of the HI shell (thus supporting the latter hypothesis of origin of the pulsar).

The coincidence in both coordinate position and distance of the pulsar PSR0740-28 and the neutral hydrogen shell GS241-01+15, in conjunction with the presence of two nearby non-thermal radio sources, G242.5-3.5 and G240.8-1.2, is strong support for the physical relationship of these two objects. It is not yet clear whether the pulsar and the H I shell originated in a single supernova event, or whether formation of the HI shell may have preceded the birth of PSR0740-28. The current data seem to indicate that PSR0740-28 may be the remnant of a second-generation supernova, in support of the conclusions of Morris et al.2 regarding the relationship between the pulsar and the non-thermal source G242.5-3.5. Future observations, particularly proper motion studies, should clarify the situation.

We thank A.S. Wilson for useful discussions and D. Klinglesmith for arranging use of the Interactive Astronomical Data Analysis Facility of the Goddard Space Flight Center. Partial support of this research was provided by the NSF through grant AST 80-21283 to Professor F. J. Kerr of the University of Maryland. The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with NSF.

Received 21 September 1981; accepted 19 January 1982.

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Overshoots in planetary bow shocks

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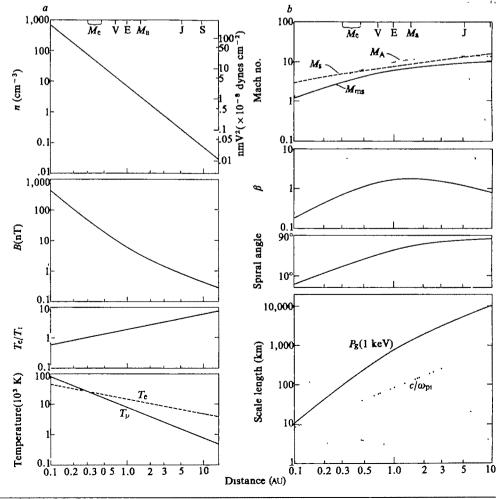
An overshoot in the magnitude of the magnetic field is shown here to be a consistent feature of supercritical collisionless shocks, Mach number ≥3, throughout the Solar System. Although the variability of the solar wind at 1 AU allows us to study the Earth's bow shock over a wide range of B, the ratio of thermal pressure to magnetic pressure, the Mach number of the solar wind at 1 AU varies over a relatively narrow range. Data from Jupiter and Saturn allow us to study the bow shock at high Mach numbers rarely, if ever, observed at Earth. These combined planetary data show that the planetary bow shocks, at least as characterized by their overshoots, form part of a continuum, differences being dependent mainly on the varying solar wind conditions at each of the planets. The overshoot magnitude is found to increase both with β and magnetosonic Mach number. However, it is the radial variation of Mach number which causes the overshoot in the jovian and saturnian bow shocks to be much greater than in the terrestrial shock.

Collisionless bow shock waves exist in front of each planet probed by planetary spacecraft so far. These shocks both deflect the supersonic, or supermagnetosonic, solar wind around the planets and heat the solar wind. Part of the incoming solar wind is accelerated or 'reflected' by the shock back along magnetic field lines towards the Sun. This reflection process has been described geometrically by Sonnerup¹ but no detailed mechanism has yet been postulated to reflect the observed small fraction of the incoming ion beam. The change in the solar wind

plasma across the bow shock can be understood in magnetohydrodynamic formalism according to the Rankine-Hugoniot equations². However, these equations do not describe the plasma physical processes by which the heating and deflection occur, the relative energization of ions and electrons, the heat flux represented by the 'reflected' ion beams, or the fine scale structure of the shock on scale lengths comparable to an ion gyro-radius or less. Investigation of the microphysics of collisionless shocks is necessary to understand these processes and to learn why in collisionless shocks a small fraction of the particles receives a large fraction of the energy. This behaviour is important, not just for understanding the creation of energetic particles in the Solar System, but also for understanding the generation cosmic rays outside the Solar System. It is quite likely that stellar, interstellar and even intergalactic shocks are producing cosmic ray beams analogous to the ion beams in planetary foreshocks.

Because of the dispersive nature of waves in a plasma one would expect damped wave trains either upstream or downstream from the shock front². What is generally observed in planetary shocks is a single approximately critically damped wave cycle or overshoot in the magnetic field strength. This overshoot was first noted by Heppner et al.³ in OGO 1 magnetic field records and later reported in ISEE 1 and 2 data^{4,5}, in which it was shown that while electron acceleration is coincident with the overshoot in field magnitude, ion thermalization is not complete until the overshoot has finished and the field returns approximately to its equilibrium or Rankine-Hugoniot value. There is reason to believe this overshoot is associated intimately with the ion 'reflection' process for unpublished ISEE magnetic field data indicate a scale length for the overshoot comparable to the ion gyro-radius, and the plasma data show ions reflected by the overshoot. These observations are supported by computer simulations which also demonstrate the ion gyration nature of the overshoot6.

1 Typical solar parameters as a function of heliocentric distance. a, Number density and dynamic pressure (top panel), magnetic field strength, ratio of electron to proton temperature, and the electron and proton temperatures. b, Mach numbers, Alfvén, sonic and magnetosonic (top panel), β (ratio of thermal to magnetic pressure), the spiral angle (angle between interplanetary field and solar wind flow) gyroradius of 1-kev proton and the ion inertial length. The labels at the top give the planetary positions.



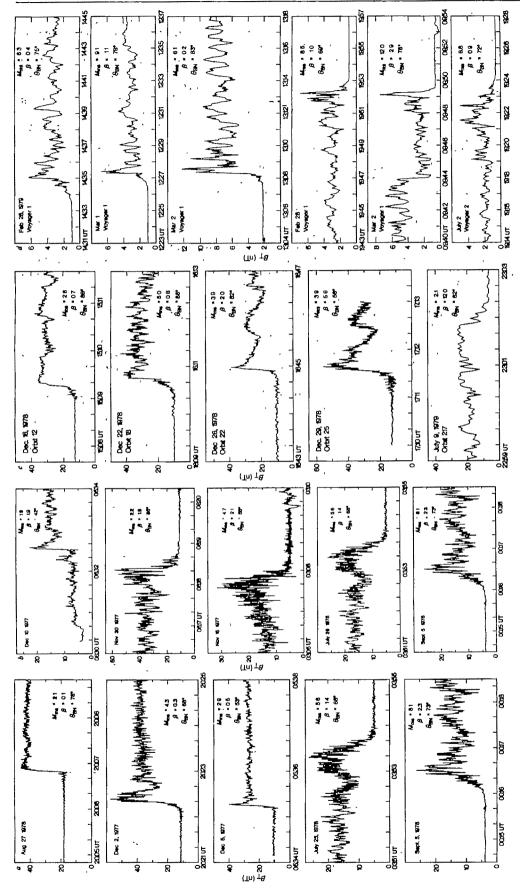


Fig. 2 Magnetic field profiles of the terrestrial bow shock as observed by the ISEE 1 magnetometer in a variety of solar wind conditions. a, β increases from top to bottom. b, Magnetosonic Mach number increases from top to bottom. c, Magnetic field profiles of the Venus bow shock in a variety of solar wind conditions. The top two panels contrast shocks for different Mach number. The middle two panels contrast shocks for differing β , d, Six crossings of the joynan bow shock as detected by Voyagers 1 and 2.

Although the existence of the overshoot in the terrestrial shock has long been known, its parametric variation with solar wind conditions has not been studied nor has its possible importance in the ion reflection mechanism been appreciated. We now consider the parametric variation of the overshoot with solar wind conditions by using both terrestrial and planetary

bow shock data. At the Earth the day-to-day variability of the solar wind and variation of the angle between the solar wind flow and magnetic field and the shock normal along the curved bow shock provide a wide range of plasma conditions. On the other hand, despite this variability the range of plasma conditions at 1 AU is limited and examples of shocks in some interesting

portions of parameter space, especially at high Mach numbers, are rare. This difficulty can be partially overcome through studying the bow shocks of the other planets as the variation of the solar wind properties with heliocentric distance places each of the planets in a different average environment.

This variation is illustrated in Fig. 1a, in which the dependence of various solar wind parameters is plotted as a function of heliocentric distance. The curves were constructed by taking estimates of typical values at the Earth (J. T. Gosling, personal communication), assuming a constant solar wind speed and scaling density as r^{-2} , magnetic field strength as r^{-1} ($r^{-2}+1$)^{1/2}, proton temperature as r^{-1} and electron temperature as $r^{-1/2}$. In addition to these basic parameters we have studied the radial dependence of a set of physical quantities derived from them which have proved useful in classifying shock structure. These quantities are plotted against heliocentric distance in Fig. 1b. Included are plots of β , the spiral angle of the interplanetary field, the length scales of the ion gyroradius $(\rho_{\mathbf{z}})$ and ion inertial length (c/ω_{pl}) and three Mach numbers. The Mach numbers are the Alfvén Mach number, the ratio of the solar wind velocity with respect to the Alfvén velocity, the sonic Mach number, the ratio of the solar wind velocity to the sound velocity and the magnetosonic Mach number, the ratio of the solar wind velocity to the magnetosonic velocity, or fast mode velocity. The magnetosonic velocity is calculated for propagation perpendicular to the magnetic field and is the square root of the sum of the squares of the Alfvén and sound velocities. All of the Mach numbers rise monotonically by factors ~3 from the orbit of Mercury to that of Saturn.

If the magnetosonic velocity were corrected for its dependence on angle of propagation to the magnetic field, the corrections would be small, especially at the larger heliocentric distances where the spiral angle of the interplanetary magnetic field becomes more perpendicular to the solar wind velocity. β first increases and then decreases with increasing distance, rising to its maximum just beyond the orbit of the Earth. The ion inertial length and gyroradius increase by one and two orders of magnitude respectively from Mercury to Saturn.

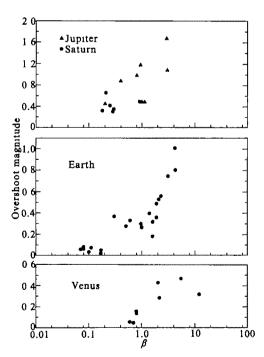


Fig. 3 Strength of the overshoot in the magnetic field immediately behind the shock as a function of β for Venus, Earth, Jupiter and Saturn. The overshoot magnitude is defined to be the maximum field strength minus the equilibrium average field strength some distance behind the shock normalized by this equilibrium average. The scale for the upper panel is double that of the bottom two panels.

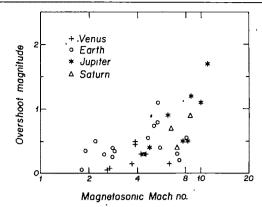


Fig. 4 The magnitude of the overshoot as a function of magnetosonic Mach number.

Experimental evidence that these predicted changes in plasma environment from planet to planet are reflected in the evolution of bow shock structure with heliocentric distance has been provided by Scarf et al.⁷ in their studies of the plasma wave spectra associated with various planetary bow shocks. The very low frequency spectrum across the bow shock of Saturn is distinctly peaked, whereas at Venus it decreases monotonically with increasing frequency. Data at the Earth and Jupiter show intermediate behaviour. Scarf et al.⁷ hypothesize that the nature of the bow shock undergoes a transition in the neighbourhood of a Mach number of 7–10.

We use magnetic field data obtained at Venus, Earth, Jupiter and Saturn to study the heliocentric evolution of bow shock overshoot and its dependence on solar wind parameters. We do not use data obtained at Mercury or Mars because of the paucity and/or unavailability of high resolution data. Figure 2a shows samples of ISEE 1 magnetic profiles across the terrestrial bow shock in a variety of solar wind conditions (Los Alamos Space Plasma Group, personal communication). On 27 August 1978 at low Mach number and β there is little if any post-shock overshoot in the magnetic field. However, at higher Mach number and β the overshoot is quite prominent. These shocks have been arranged in order of increasing β from top to bottom.

There is a variety of shock structure; the field can at times be quite irregular and the overshoot can be difficult to define unambiguously or to distinguish from 'normal' magnetosheath fluctuations. We have chosen a simple empirical parameterization of the overshoot and define the magnitude of the overshoot to be the difference between the equilibrium or average downstream field strength and the maximum field strength just behind the shock normalized by the equilibrium average field strength.

Figure 2b shows a set of shocks similar to those in Fig 2a arranged in order of increasing magnetosonic Mach number with β held fixed at \sim 2. There seems to be an evolution in structure with changing Mach number but the evolution is partly masked by our inability to fix the angle between the upstream field and the shock normal. Furthermore, despite our examination of >1 yr of ISEE data we have been unable to separate clearly Mach number effects from β effects. Indeed the bottom two examples in Fig. 2a, b are the same cases.

Similar sets of shocks with contrasting solar wind parameters chosen from Pioneer Venus Orbiter data⁹ are shown in Fig. 2c. Solar wind conditions at Venus are similar to those seen at Earth, and the bow shock structure is similar although weaker than the terrestrial shock¹⁰. The top two panels of Fig. 2c contrast the bow shock at fixed β and angle between the magnetic field and shock normal but with different Mach number. The second pair show the increase in turbulence associated with an increase in β . The bottom panel shows the case with a very high β . However, the low data rate at this time obscures the detailed structure of the shock.

At Jupiter and Saturn we expect lower β than at 1 AU but higher Mach numbers. This is confirmed in Fig. 2d which shows six jovian bow shocks obtained in only a few days as observed by

the GSFC Voyager magnetometer and their associated plasma parameters. The plasma data necessary for the calculation of Mach number and β were provided by the MIT plasma group. It is evident that the jovian overshoots are much bigger than their terrestrial counterparts. Data for the bow shock of Saturn have recently been published11 and will not be repeated here. The Mach number during the Pioneer 11 passage by Saturn was less than at Jupiter during the Voyager encounters. However, it was still much greater than normally observed at 1 AU.

The two parameters which seem to control the structure of the shock in Fig. 2 are β and Mach number. Thus we have taken a sample of the bow shocks at Venus, Earth, Jupiter and Saturn including those shown and examined the overshoot as a function of β and Mach number. Figure 3 shows the amplitude of these overshoots plotted against β for Venus and the Earth separately and Jupiter and Saturn combined. The scale for Jupiter and Saturn is twice that for Venus and Earth so that there is a different variation at the outer planets. However, qualitatively there is a clear trend of increasing overshoot with increasing β for all the planets.

Figure 4 indicates that the difference between the overshoots at the various planets is due to the difference in Mach number.

Received 27 July, accepted 9 December 1981

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Plotting all the planetary data as a function of magnetosonic Mach number suggests that the trends for each planet coincide. The principal difference being the range of Mach numbers at each planet. Clearly there is also a dependence on β , but the range of B is similar at each planet. We have also checked to see whether the apparent β and Mach number dependences could have been caused by a correlation between β and Mach number but there is, in fact, little correlation between these two parameters, except in the terrestrial data set by itself in which high Mach numbers were always accompanied by high betas.

We conclude that the bow shock structure, notably the magnitude of the overshoot in field strength post-shock, depends on both β and magnetosonic Mach number. This dependence seems to be continuous and we find no evidence for a sudden change in structure at the highest Mach numbers at least as observed in the magnitude of the magnetic overshoot.

We thank S. J. Bame, A. Barnes, H. Bridge, J. T. Gosling, A. J. Lazarus and J. Scudder for the plasma data. The Voyager magnetic field (N. Ness, P.I.) were provided by the National Space Science Data Center. This research was supported by NASA under contracts NAS2-9491 and NAS5-25772 and under grants NGL 5-007-190 and NAGW 162.

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Magnetoid interaction with surrounding stars

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Lugg¹ has recently analysed a magnetoid—a supermassive rotating highly magnetized star²⁻⁵—situated at the centre of a compact galactic nucleus in the belief that previous investigations had taken no account of interaction with neighbouring stars. Such an interaction has been widely discussed⁶⁻¹¹, but there has been no confirmation of the main conclusion of ref. 1 that these stars can disrupt a magnetoid in as short a time as 104 yr. Here we demonstrate that this conclusion is erroneous.

On penetrating a magnetoid, stars greatly decelerate and thus, in gradually losing their kinetic energy, settle to the magnetoid's centre. Although this process is accompanied by numerous complex phenomena (such as shock-wave generation, ablation, interaction of radiation with star atmospheres and partial mass loss by stars) we shall assume, as in ref. 1, that a supermassive star (superstar) supported mainly by radiation pressure will be formed eventually from the captured stars at the magnetoid's centre. Lugg believes that such a superstar will collapse as soon as its mass exceeds $10^5 M_{\odot}$. However, this value for the critical mass used in ref. 1 has been taken from the theory for an isolated superstar whereas in the present case the central superstar is under an appreciable external pressure. We shall show now that in these conditions the critical mass can be much greater than $10^{\circ} M_{\odot}$.

In the first post-newtonian approximation, the equilibrium energy of a superstar with mass M and radius R experiencing an external pressure $P_{\bullet}(R)$ on its surface, takes the form

$$\mathscr{E}_{\bullet} = -(3\gamma - 4)E_{\text{th}} + 4\pi R^{3}P_{\bullet} + \int 4\pi r^{2}P_{\bullet}(r) dr + \zeta_{1} \frac{G^{2}M^{3}}{R^{2}c^{2}}$$
 (1)

where $(3\gamma - 4) = \beta/2$, $\beta = (gas pressure/total pressure) =$

4.3 $\mu^{-1}(M/M_{\odot})^{-1/2}$ where μ is the mean molecular weight per particle. It is convenient to approximate $P_{\mathbf{s}}(R) \sim R^{-q}$ with q =constant. Then, considering the superstar as a polytrope of index three $(\zeta_1 = 5.06)$ we have in the critical state $(d\mathcal{E}_o/dR = 0)$:

$$R_{\rm cr} = \frac{6.8}{\beta + 2(4 - q)\alpha} R_{\rm S} \tag{2}$$

 $R_{\rm S} = 2 \, GM/c^2$ being the Schwarzschild radius; $\alpha = P_{\rm s}/\tilde{P} = 94 (M_{\rm m}/M)^2 (R/R_{\rm m})^4$; $M_{\rm m}$ and $R_{\rm m}$ being mass and radius of the magnetoid embracing the superstar. If $M \ll M_m$, then $q \approx 0$ in a good approximation, so that

$$\frac{R_{cr}}{R_{s}} = \begin{cases} 1.58 \ \mu (M/M_{\odot})^{1/2} & \text{at } \beta \gg 8\alpha \\ 0.85\alpha|_{R=R_{cr}}^{-1} & \text{at } \beta \ll 8\alpha \end{cases}$$
(3)

On comparing the upper line in equation (3) (external pressure is negligibly small) with the lower line (external pressure is substantial) it is clear that in the latter case the value of the critical mass, M_{cr} , is much greater. We calculate it in a specific case when the superstar is embedded in a magnetoid of mass case when the superstar is embedded in a magnetoid of mass $M_{\rm m}=10^8 M_{\odot}$ and radius $R_{\rm m}=10^{16}\,{\rm cm}$ (the same magnetoid parameters are used in ref. 1). In this case we have $\alpha=1.3\times10^5 (M/M_{\odot})^{2/5}$ at $R=R_{\rm cr}$, and the external pressure term is dominating in equation (3) if $M>1.3\times10^5\,\mu^{-10/9}M_{\odot}$. Making R_{cr} equal to the radius, R_{b} , of the superstar which has reached the hydrogen burning state:

$$\frac{R_{\rm b}}{R_{\rm S}} = 6.84 \times 10^5 \left(\frac{M}{M_{\odot}}\right)^{-0.535} \left[XZ(1+X)\right]^{0.069} \tag{4}$$

(ref. 12) we get for the standard population I composition (X = 0.70, Z = 0.03)

$$M_{\rm cr} = 0.67 \times 10^7 M_{\odot} \text{ for } M_{\rm m} = 10^8 M_{\odot}, R_{\rm m} = 10^{16} \text{ cm}$$
 (5)

At this stage, the radius of the superstar of the critical mass $R_b = 1.2 \times 10^2 R_s = 2.4 \times 10^{14} \text{ cm}$, that is $R_b / R_m \sim 2 \times 10^{-2}$. Such a small value of this ratio implies that the simplifying assumption q = 0 used above to derive equation (3) is well satisfied even for masses as large as M_{cr} given by equation (5).

We see that M_{cr} is much greater than the value $10^5 M_{\odot}$ used in ref. 1. In other words, the central superstar accumulated from the remnants of captured stars will reach the hydrogen burning

stage even without noticeable influence of external pressure if its mass $M < 2 \times 10^5 M_{\odot}$ or due to the pressure if $2 \times 10^5 M_{\odot} \le M \le$ $0.7 \times 10^7 M_{\odot}$. But in neither case does this superstar collapse. Moreover, the nuclear burning gives the superstar a main sequence lifetime of as long as $10^6\,\mathrm{yr}$, independent of its mass and with only a weak dependence of its initial heavy element content¹² unless the hydrogen abundance of the captured stars is abnormally low.

Lugg¹ has found that the mass accumulated in the central superstar is $M \ge 10^5 M_{\odot}$, but such a large value is a result of two basic assumptions made in ref. 1. First, it was assumed that the star cluster surrounding a magnetoid has a mass $M_c \ll M_m$. However, there is considerable evidence in favour of an opposite inequality both for active galactic nuclei and for 'quiescent' ones, such as the nucleus of our Galaxy (see below). As a result, the cross-section for interaction of stars with a magnetoid is equal to

$$\sigma = \pi R_{\rm m}^2 (1 + 2GM_{\rm m}/R_{\rm m}v^2) \approx 2\pi R_{\rm m} r_{\rm c} (M_{\rm m}/M_{\rm c})$$

that is it is smaller than the value in ref. 1 by a factor $M_{\rm m}/M_{\rm c}$ ($r_{\rm c}$ is the core radius). Furthermore, the value $M_m = 10^8 M_{\odot}$ used in ref. 1 should be attributed to a magnetoid which has the characteristics of a quasar but not of an active galactic nucleus, and certainly not that of our own Galaxy. From the requirement that the non-thermal luminosity ($\leq 10^{44}-10^{45}$ erg s⁻¹) observed for active galactic nuclei should be less than or equal to the Eddington luminosity of a hot magnetoid it follows that $M_{\rm m} \leq 10^6$ - $10^7 M_{\odot}$ in active nuclei. Meanwhile the collision rate of stars with the magnetoid is $f = \sigma n_c v \sim M_m R_m (r_c/M_c)^{1/2} n_c$, n_c being the number density of stars in the core. Taking into consideration that $R_m \sim M_m$ (ref. 5) we have $f \sim M_m^2$, that is f decreases greatly as M_m diminishes.

A comparatively low value of M_m is expected in active galactic nuclei, making it reasonable for an inequality $M_m \ll M_c$ (opposite to that used in ref. 1) to take place there. This agrees well with the fact that the velocity dispersion of stars in the cores of both M31 and our Galaxy is as low as $v \approx 200 \text{ km s}^{-1}$ being determined by the gravitational field of an extended mass $M_c \sim 10^7 - 10^8 M_{\odot}$, and not by that of a point (magnetoid) mass of value $M_{\rm m} = 10^8 M_{\odot}$ which gives $v \approx 600 \, {\rm km \, s^{-1}}$. Therefore, if the activity of a Seyfert nucleus is conditioned by the central magnetoid acting within a normal stellar core, inequality $M_{\rm m}$ \ll M_c will be satisfied there. This inequality will only be stronger if the Seyfert phenomenon originates in a galaxy having a more powerful spherical component. (Note that there is strong evidence for such an inequality within the framework of black hole models: see refs 13, 14).

The lower value of M_m and inequality $M_m \ll M_c$ in active nuclei both suggest that the total mass of stars which succeed in sticking inside the magnetoid in the time of refilling of low angular momentum orbits should be considerably less than the value $\ge 10^5 M_{\odot}$ of ref. 1. Even if this mass were much greater (for example, because of collective gravitational effects) but less than M_{cr} given above, it would lead not to its collapse but to a rather slow evolution on the nuclear time scale of about 106 yrcomparable with the lifetime of an isolated magnetoid. Therefore, the conclusion in ref. 1 about the rapid disruption of a magnetoid by the surrounding stars is wrong.

Nevertheless, interaction of stars with the magnetoid has several important and interesting aspects (especially from an observational point of view) mentioned in refs 6-11. Note that we do not regard a magnetoid as the only possible 'engine' responsible for the activity of quasars and nuclei of galaxies. The advantages and difficulties of three possible sources of activitya massive black hole, a magnetoid and compact star cluster-are discussed in detail elsewhere 15-18. The observational tests proposed in refs 15-18 would help to create a realistic theory of the engine.

Received 31 July: accepted 3 December 1981

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Initial microbiological response in lakes to the Mt St Helens eruption

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No published reports exist on the early stages in the establishment and succession of microbial communities and associated chemical and geochemical transformations in aquatic environments shortly after a volcanic event. Much of our understanding of volcanic lakes has come from studies carried out years to centuries after the eruption, when the lakes were already stabilized. Here we document the chemical and microbiological responses of lakes and hydrothermal environments in the blast zone near Mt St Helens 3-4 months after the eruption of 18 May 1980. Within weeks of the eruption, the impacted lakes became anaerobic, ultraeutrophic and developed extensive populations of bacteria, some of which were not common to those environments. By August 1980 both assimilative and dissimilative nitrogen cycle reactions were regulating the extent of chemosynthetic, photosynthetic and heterotrophic activities occurring in the lakes. Recovery of the lakes to their normal oligotrophic state will result in part from oxygen production by photosynthesis and anaerobic oxidation of reduced metals, gases and organic compounds coupled with denitrification.

There are numerous oligotrophic sub-alpine lakes surrounding Mt St Helens (Fig. 1), which were dramatically altered by the 18 May 1980 eruption. Some lakes and streams were reshaped, new lakes and numerous hydrothermal seeps emerged. Aquatic environments in the blast zone received massive quantities of ash, soil, wood debris and pyrolized soluble organics from the destroyed forest. Previously oligotrophic lakes became ultraeutrophic and anoxic.

The differences in the chemical and biological characteristics of the Mt St Helens lakes in August and September 1980 compared with before the 18 May eruption generally reflected the degree of impact. The lakes can be grouped into three categories based on impact: (1) those receiving only ashfall, such as McBride, June and Merrill; (2) lakes in the blowdown or scorch zones, such as Ryan, Boot, Meta, Panhandle, Venus, Island, Hanaford, Fawn and St Helens; and (3) lakes affected by mud and debris flows, including Spirit, North and South Coldwater, Castle Creek and other new lakes formed by mudflows damming the Toutle River. The heavily impacted lakes near the crater and in the path of mudflows soon became anaerobic due to increased microbial activity fuelled by the high concentrations of dissolved organic carbon (DOC), sulphur compounds and metals entering the lakes, with the result that massive levels of reduced metals, gases and low molecular

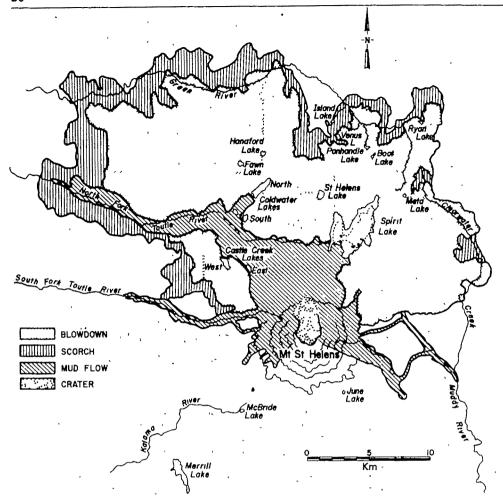


Fig. 1 Map of the lakes in the region surrounding Mt St Helens, showing areas of blowdown, scorch and mudflows. The lakes outside these zones received ashfall but were not in the blast zone.

weight organic compounds were mobilized into the water column. Lakes in the blowdown or scorch zones of the blast area maintained aerobic surface layers over anaerobic hypolimnia. A thermocline was present in lakes in blowdown and scorch zones. Lakes outside the blast zone, which received only ash, have changed the least and remain oxygenated.

All lakes near the volcano have received periodic input of volcanic ash, of dacitic composition, consisting primarily of silica

and aluminium oxides^{1.2}, and mainly of sand size³. Elements of potential biological significance in the ash include Fe (3.7%), P (0.10%), Mn (0.07%) and Mo (1.9 p.p.m.). Leaching experiments using a water/ash ratio of 10:1 in aerobic conditions yielded no decrease in pH and released into solution the following nutrients (in p.p.m.): SO_4^{-2} (57), PO_4^{-3} (3), Mn (0.4), Fe (0.01) and Mo (>0.005)^{1.2}. Most of the constituents of the ash are insoluble in aerobic conditions.

Table 1 Physical and chemical characteristics of lakes and hydrothermal seep in the blast zone of Mt St Helens

MIN		,		Lakes	199171. ·		
Messurement	Ryan (surface)	Ryan (3m)	Ryan (6m)	Spirit (surface)*	N. Coldwater (surface)	N Coldwater (seep)	McBride (surface)
Distance from crater (km)	19	19 '	`19	5	11	11	9
Depth (m)	['] 6	6 '	6	15	34	0.2	7
Area (ha)	1.6	1.6	1.6	700	100	1–3m²	3.6
Temperature (°C)	20	14	. 7	19 -	22	72-80	16
pΗ	6.88	6 55	6.43	6.80	6.21	5.35	7 26
O ₂ (mg l ⁻¹)	1.43	. 0 00	0.00	0.00	1.61	0.00	8.72
ΣCO ₂ (mM)	0.25	0.55	1.58	2 05	3.60	ND	0.14
$Fe^{2+} (mg l^{-1})$	0.18	1 21	2.57	1.08	6.27	9 26	0.00
$Mn^{2+} (mg l^{-1})$	0.71	ND	ND	4 48 -	3.35	ND	ND
PO ³ -P (mg l ⁻¹)	0 014	0.020	0.021	0.031	0.022	0.790	ND
Total P (mg l ⁻¹)	0.027	0 045	0.027	0 042	0.043	1.556	ND
$NO_2^- + NO_3^- (mg l^{-1})$	0.010	0.000	0.000	0 000	0.000	0.020	0.011
$NH_{4+} (mg l^{-1})$	0.012	. 0 009	0.031	0 039	0.000	0.316	ND
DON $(mg l^{-1})$.	0.195	0 087	.0.123	0.213	0 690	4.204	ND
DOC (mg l ⁻¹)	10.2	16.3	46 4	51.1.	114	4,285	3.7
DOC/DON	52	187	377	240	165	1,019	ND
N_2O (nM)	` 8.8	7.0	0.6	7.4	6 1	18.8	9.4
H_2 (nM)	41 .	5.0	·· 1.0	20	20->200	15 5	9.0
CO (nM)	238	2 58	87	193	100->150	395	54
СН, (μΜ)	5.2	8.2	12.3	15.4	28->250	1-6 3	1.3

Data are from sampling on 10 August, 19 August, or 11 September 1980. The methods used for chemical measurements are reported in the references given in parentheses: O₂ (16), CO₂ (17); Fe²⁺ (18); Mn (19); PO₄P, NH₄, NO₂, NO₃ and DON (20); total P (21); DOC (22); N₂O (23); and H₂, CO and CH₄ (using a modification of the procedures in ref. 24). ND, no data.

^{*}Surface water temperature at Spirit Lake on 19 May 1980, the day after the major cruption, was 34 °C (T. Casadevall, personal communication). Surface temperature measured on 4 April 1980 was 4 °C (R. C. Wissmar, personal communication). Historical data indicate that typical temperatures for the middle of May would be ~10 °C. The surface concentration of DOC on 4 April 1980 was 0.83 mg Cl⁻¹ in Spirit Lake (R. C. Wissmar, unpublished data) Surface values for DOC on 30 June, 11 August and 19 August 1980 were 39.9, 39.9 and 51.1 mg Cl⁻¹ respectively.

Table 2 Microbiological characteristics of lakes and hydrothermal seep in the blast zone of Mt St Helens

					Lake	s			
Measurement	Ryan (littoral)	Ryan (midsurface)	Ryan (3m)	Ryan (6m)	Spirit (surface)	N. Coldwater (surface)	N. Coldwater (stream)	N. Coldwater (isolated pond)	N. Coldwater (hot water seep)
Total no. bacteria per ml Total no. sulphur/Mn- oxidizing bacteria	3.0×10^8	3.0×10^{7}	1.5×10 ⁷	6.4×10 ⁶	4.2×10 ⁸	$3.4-7.0 \times 10^7$	1.2×10 ⁸	4-6.7×10 ⁷	9.1×10 ⁶
per ml	$0.8-1.0\times10^{6}$	5.0×10^{4}	2.0×10^{6}	2.0×10^{6}	5.0×10^{6}	$3-3.4 \times 10^{5}$	3.0×10^{6}	$2-5.5 \times 10^{2}$	ND
N ₂ fixation									112
(light aerobic nM N ₂)	$(cm^{-2}h^{-1})$	$(l^{-1} h^{-1})$	ND	ND	ND	ND	ND	NDet	NDet
N ₂ fixation (dark anaerobic									
$nM N_2 l^{-1} h^{-1}$	140	130	180	220	400	180->230	170	NDet	NDet
Denitrification		Value 200	C-C-1/5()		1,000		***	11001	
$(\mu M N_2 O l^{-1} day^{-1})$	<1	<1	10	30	36	50	70	12	5
Nitrification					-5/4/				
$(\mu g NO_2 l^{-1} day^{-1})$	12	2	< 0.1	< 0.1	< 0.1	< 0.1	0.4-3.0	< 0.1	< 0.1
Presence/absence of:							200.500		
cyanobacteria	+	+	-	-	+	-	ND	_	
algae + diatoms	+	+	-	_	+	+	ND	_	

Data are from sampling on 10 August, 19 August, or 11 September 1980. Total bacterial counts were determined using epifluorescent microscopy²⁵. Manganese-and sulphur-oxidizing bacteria were counted using a medium of the following composition (in g): NaCl, 2.6; KCl, 0.88; MgCl₂, 0.56; MgSO₄, 0.76; CaCl₂, 0.1; Na₂HPO₄, 9; KH₂PO₄, 1.5; NaNO₃, 1.0; Na₂S₂O₃, 5; FeSO₄·H₂O, 20×10⁻³; MnSO₄·H₂O, 150×10⁻³; Bacto yeast extract (Difco), 0.01; trace element solution according to ref. 26, distilled water to 11 and adjusted to pH 6.8. Bacto purified agar was dialysed in distilled water to remove soluble organics and 12 g l⁻¹ used. Manganese oxidation was measured using a benzidinium reagent as described in ref. 27. The procedures used for microbiological activity measurement were: N₂ fixation, ref. 28; denitrification, ref. 29; nitrification, ref. 30. The rates of N₂ fixation are only apparent. The acetylene reduction method is an indirect assay for fixation, and the rates have not yet been verified with ¹⁵N₂. Conversion to nanomoles of N₂ fixed was done using an assumed theoretical ratio of 3.0 mol of ethylene produced per mol of N₂ fixed. The theoretical ratio has been reported to vary from 2.0 to 25 (ref. 31). Recently a conversion factor for an anaerobic bacterial population, *Microcoleus chthonoplastes*, in solids was given³² as 5.4. Although the rate of N₂ fixation may be about double the actual rate, it is still much greater than previously reported anaerobic dark fixation rates in a water column. Blue-green algae isolated from lakes in the blast zone included *Pseudanabaena*, *Anabaena* and *Oscillatoria*. Other algae found included *Nitschia*, *Euglena*, *Spirogyra* and an encysting motile, unicellular green alga. ND, no data; NDet, not detected.

In the blast zone, the tremendous heat associated with the eruption (200-350 °C) pyrolized much of the coniferous forest foliage. Throughout the mudflow, blowdown and scorch zones, the concentration of DOC and particulate organic carbon (POC) increased greatly, and the temperature was higher than in nearby lakes outside the blast zone (Table 1). DOC in Spirit Lake increased from $<1.00 \text{ mg C l}^{-1}$ to $>30 \text{ mg C l}^{-1}$, and in North Coldwater and Castle Creek Lakes DOC exceeded 100 and 200 respectively. Heterotrophic microbial activity coupled with the decreased solubility of oxygen at the elevated temperatures quickly consumed the available O_2 , On 30 June 1980, in Spirit Lake, dissolved O_2 was 2.35 mg l^{-1} at 0.25 m (R. C. Wissmar, unpublished data). Throughout August and September no O2 was detected at 0.2 m in Spirit, Castle Creek and North Coldwater Lakes. Lakes outside the path of mudflows but in the blast zone were anaerobic at depth but retained a surface layer with some dissolved O2; those receiving only ashfall were oxygenated throughout.

During the summer and early autumn of 1980, we made four 1-day expeditions to lakes and hydrothermal seeps in the blast zone of Mt St Helens. Ryan, Spirit and North Coldwater Lakes, and the hydrothermal seeps near North Coldwater, were sampled most intensively. The water at all sites was light brown to black in colour due to high levels of sulphides and DOC (Fig. 2). The sediments were actively degassing, giving off methane and other gases, so that in some lakes, such as North Coldwater, the entire surface was continuously bubbling (see Fig. 2). The

bubbles in the lake and at isolated puddles at North Coldwater were collected and analysed and found to consist of $\sim 50\%$ CH₄, 50% CO₂ and trace levels of H₂, CO and N₂O. At the hydrothermal seeps, a black odoriferous liquid with temperatures up to 80° C was emerging and multi-coloured organic films (oil slicks) formed on the surface. The films were periodically broken apart due to violent degassing of, primarily, CH₄, CO₂ and CO (Table 1).

The threefold increase in carbon, phosphorus, sulphur, iron and manganese concentrations, the presence of dissolved gases and the microbial activities in the water suggest that nitrogen cycle processes, including nitrogen fixation, nitrification and denitrification, are important (Tables 1, 2). Dissolved organic nitrogen (DON) concentrations remained low, due to the low C/N ratio of the conifer remains blasted into the lakes and the paucity of nitrogen in volcanic ash4.5, and resulted in low inorganic nitrogen (NH₄, NO₂, NO₃) concentrations, high rates of nitrogen fixation and high DOC/DON ratios. For example, Ryan Lake, which maintained an oxygenated surface layer and algal flora, had a C/N ratio of 52 at the surface but 377 near the bottom. Spirit and North Coldwater Lakes had ratios of 150-250 while that at the hydrothermal seeps exceeded 1,000. The nitrogen concentrations, relative to carbon, phosphorus and sulphur, suggested a nitrogen limitation.

Such a notion was supported by the high rates of phototrophic N_2 fixation and anaerobic dark N_2 fixation (Table 2). Anaerobic dark N_2 fixation was ≤ 230 nM N_2 l⁻¹ h⁻¹ in the surface waters

Fig. 2 Photographs of a, isolated pond near North Coldwater Lake, ~1 week after being filled by rain and showing extensive outgassing and dark black coloration of the water (pond diameter is ~1.5 m) and b, 80 °C hydrothermal seep near North Coldwater creek. Surface of the seep is covered over by a dark, 1-2 cm dark organic sulphide crust which looks like an oil slick.





of North Coldwater Lake. Brezonik and Harper reported a maximum rate of 11 nM l⁻¹ h⁻¹ in one of three lakes with extensive anoxic environments⁶. Such high rates of fixation have not previously been reported in these conditions, and represent a major source of reduced nitrogen to the lakes in the blast zone of Mt St Helens.

The number of bacteria in the lakes and seeps in the blast zone routinely exceeded 10⁸ organisms per ml (Table 2). The manganese/sulphur oxidizing bacteria were repeatedly detected in excess of 106 per ml, a value comparable with the total bacterial counts in eutrophic lakes which generally range from 10⁶ to 10⁷ organisms per ml (ref. 7). Very active methanogenesis occurred in the anaerobic sediments of all lakes and hydrothermal seeps. In addition to ebullition of CH4-rich bubbles at all sites, the dissolved levels ranged from 5 to $>250 \mu M$. High levels of CO, H₂ and N₂O were also detected in all impacted lake waters (Table 1). The exceedingly high concentrations of CO in lake surface waters at ambient temperature and North Coldwater hydrothermal seep waters have not been previously reported. The microbiological processes involved in CO production are not well understood.

Denitrifying bacteria were present in all but the oxygenated surface waters at Ryan Lake, even at 78°C at the North Coldwater seep. The manganese/sulphur oxidizing bacteria were the most abundant denitrifying bacteria, but unlike most stratified and meromictic lakes, where the metal-oxidizing bacteria are detected only at the oxycline, in Mt St Helens lakes the denitrifying metal oxidizers oxidize throughout the anaerobic water column and thus resemble estuarine sediment denitrifiers. Whereas denitrification was occurring in all aquatic environments except the surface waters at Ryan Lake, significant nitrification was limited to the oxygenated zone at Ryan Lake with greatest activity detected in the littoral zone. apparently associated with the nitrogen-fixing cyanobacteria. Significant rates of nitrification were detected in the oxygenated stream that runs into North Coldwater Lake and it is presumed that the stream is the source of nitrate for the lake (Table 2). This interpretation is supported by the reduced rates of denitrification in the isolated pond at North Coldwater.

Overall, the numbers and activities of the bacterial community reflected the widespread anaerobic conditions in the lakes and hydrothermal seeps in the blast zone in August and September 1980. The rates of this microbial biogeochemical cycling in the waters in the blast zone near Mt St Helens rivals those normally found in estuarine muds or the sediments of eutrophic lakes⁸⁻¹⁵. We believe that the rate at which these ecosystems return to the oligotrophism existing before the 18 May eruption will be largely regulated by nitrogen cycle processes associated with bacterial and algal metabolism.

Finally, we believe that many of the heavily impacted lakes and hydrothermal seeps on Mt St Helens represent ideal experimental sites for studying anaerobic microbial processes and chemical transformations which closely resemble those suggested to characterize Archaean reducing aquatic environments.

We thank R. Kepler for graphical and technical assistance, Marie DeAngelis for performing some of the microbiological analyses and L. Gordon for use of laboratory facilities. The US Forest Service provided support and helicopter time.

Received 26 October, accepted 18 December 1981

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Speculated cause of interhemispheric oceanic oscillation

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The study of large-scale correlations of atmospheric and oceanic variables on a supra-annual scale is essential for an understanding of climatic variability. We present here new data on the mean sea-level anomalies in the eastern Indian Ocean which are consistent with supra-annual anomalies in the intensity of the main oceanic gyre, and are negatively correlated with mean sea-level anomalies in the eastern Pacific Ocean. Larger anomalies are induced in the Indian Ocean probably due to its smaller size, and zonal mean stratospheric 50 mbar geopotential heights at 10°N, 20°N and 30°N are better correlated with the Indian Ocean than with the Pacific Ocean mean sea levels. It is speculated that the reported responses are due to a random variation of the monsoonally generated heat transport between the oceans. This variability would give rise to equatorial heat anomalies in each ocean which induce the observed changes in mean sea level, and the coupled changes in the sub-tropical atmospheric circulations.

Figure 1 shows the annual mean sea levels in the Indian Ocean, based on new data for Australian ports¹. A strong coherence clearly exists between the port groups and a lag correlation analysis (J. S. Godfrey, personal communication) indicates that the level changes appear almost simultaneously (within 2 months) throughout. This coherence is similar to that occurring in the eastern Pacific Ocean between San Francisco (38°N, 122°W) and Matarani (17°S, 72°W)2, however, a clear tendency for poleward propagation of the level changes has not been established for the Indian Ocean data. Furthermore on comparing the South Australian port group (for which earlier data are also available) with a group of California ports for the period 1944-75 (Fig. 2), a remarkable negative correlation (correlation coefficient -0.69) between the two records is apparent. Typically each record shows annual mean sea-level variations of ± 5 cm on a period of 3-7 yr. A spectral analysis of each of the records gives multiple peaks of energy in this band.

These data suggest that the mean sea-level variations are due to large-scale phenomena in each ocean, linked by a common mechanism. We propose that the level changes can be interpreted as a strengthening or weakening of the major oceanic sub-tropical anticyclonic gyres, such that for a more intense gyre the anomaly of levels would be positive at the centre, and negative around the coasts, and vice versa. We develop this idea quantitatively using simple models of large-scale ocean circulation^{3,4}. We also speculate on the mechanism that initiates and

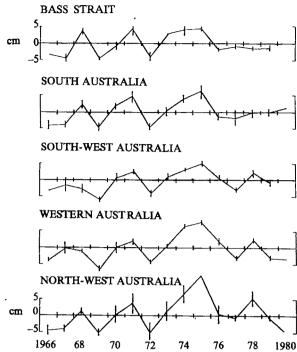


Fig. 1 Deviations in annual mean sea levels in the eastern Indian Ocean for the period 1966–80. Port groups: Bass Strait (Point Lonsdale (38°S, 145°E) and Georgetown (41°S, 147°E)); South Australia (Thevenard (32°S, 136°E), Port Lincoln (35°S, 136°E), Port Macdonnell (38°S, 141°E)); south-west Australia (Albany (35°S, 118°E) and Esperance (34°S, 122°E)); Western Australia (Geraldton (29°S, 115°E), Fremantle (32°S, 116°E) and Bunbury (33°S, 116°E)); north-west Australia (Darwin (12°S, 131°E), Port Hedland (20°S, 119°E) and Carnarvon (25°S, 114°E)). The bars indicate standard deviations between ports; a zero standard deviation is shown when data from only one port are available.

maintains the negatively correlated anomalies in each ocean. We consider first a mechanism for establishing the anomalies.

The Pacific Ocean and the Indian Ocean are connected to the north through the Indonesian archipelago, and to the south by the Southern Ocean. The northern connection lies along the track of the South-East Asian monsoon⁵, which essentially blows towards the north in the northern summer and towards the south in the northern winter, and drives a corresponding circulation in the surface waters of the passages in the archipelago. If there were a random variation in the strength of the monsoon, this would be reflected in a random variation of the heat transport between the two oceans. We suggest that this exchange of heat is the prime cause of the responses shown in Fig. 2, because a positive flux anomaly for one ocean is a negative flux anomaly for the other ocean, and vice versa. Hence the supra-annual anomalies in heat content in each ocean would be negatively correlated, their magnitudes being functions of the monsoonal variability of the previous few seasons. This conclusion is an example of the Slutsky effect⁶ whereby a moving average of a series of random numbers tends to give rise to a series which is quasi-periodic at a period approximately twice that of the length of the moving average.

The differences between the observed records are due to factors in each ocean that effectively give rise to dissimilar moving averages. Notably, the monsoonal heat flux after entering the Indian Ocean is carried westward by the south equatorial current, whereas in the Pacific Ocean its advent is opposed by the north equatorial current with the result that there is a tendency for a ponding of heat anomaly in the western Pacific Ocean. The dispersion of this pond is achieved principally through the equatorial undercurrent, and at times by a sudden discharge which results in an El Nino event⁷⁻⁹. The Indian. Ocean mean sea-level regime indeed extends into the western Pacific Ocean at least as far as Truk (7°N, 151°E)²¹. Note also that although the monsoonal heat exchange for geographical

reasons is primarily an interhemispheric exchange between the southern Indian Ocean and the northern Pacific Ocean, the effect of heat anomalies along the Equator is transmitted to the general circulations of both hemispheres.

In a baroclinic ocean the anomalies in heat content are reflected in surface level changes through the presence of a sub-surface reference level which remains approximately isobaric during the variability. Thus we hypothesize that monsoonally generated changes in ocean heat content in the equatorial regions are the primary mechanism for the induction of the observed anomalies of mean sea level. In particular, in the equatorial regions the depth of the reference level is $\sim 300 \, \mathrm{m}$ (ref. 10), from which it is deduced that temperature anomalies of $\pm 1 \, ^{\circ}\mathrm{C}$ would induce mean sea-level anomalies of $\pm 5 \, \mathrm{cm}$, an increase in level corresponding to an increase in temperature.

Consider next the implications of the persistence of the equatorial anomalies for the general circulations in the ocean and the atmosphere. First, the vorticity balance in the oceanic anticyclonic gyres requires that a cold anomaly (a negative mean sea-level anomaly) must be balanced by an increase in the anticyclonic wind stress curl. In fact, for an ocean gyre in a rectangular basin (latitudinal width L, meridional width M), which is driven by an anomalous zonal wind stress distribution, $\tau = -\tau_0 \cos \pi y/M$, an approximate expression of the anomaly of sea level on the perimeter of the basin, except along its western boundary, (assuming no change in the mass of water in the basin) is

$$\eta \sim -\frac{\tau_0}{\rho g H} \frac{f}{\beta} \frac{L}{M}$$

where ρ is the density of seawater, g is the acceleration due to gravity, $f = 2\Omega \sin \phi$ is the Coriolis parameter in which Ω is angular speed of rotation of the Earth, and ϕ is latitude, $\beta = df/dy$ (0y towards the north), and H is the depth of the reference level. Thus, assuming L = 10,000 km, M = 3,000 km, $\rho = 1,000 \text{ kg m}^{-3}$, $g = 10 \text{ m s}^{-2}$, H = 300 m, $|f| = 0.3 \times 10^{-4} \text{ s}^{-1}$, $\beta = 2 \times 10^{-11} \text{ m}^{-1} \text{ s}^{-1}$, a negative anomaly of mean sea level of 5 cm would induce an increase in the stress contrast between the trade winds and the westerlies $|2\tau_0|$ of 0.05 N m⁻². This value is of the order of 25% of the annual mean contrast of 0.2 N m⁻². It is significant that the only latitudinal variation in the (geostrophic) expression for η lies with f and H, and thus η is predicted to be of similar form at all latitudes in agreement with the data (Fig. 1). The observed similar magnitudes of the anomalies at all latitudes suggest that the reference level $H \propto f$, an inference consistent with oceanic thermocline theory¹². As L is smaller in the Indian Ocean than in the Pacific Ocean, and η is probably larger (Fig. 2), the expression for η indicates that the induced changes in τ would be more significant in the Indian Ocean than in the Pacific Ocean.

In addition to the redistribution of mass within the ocean basins due to the gyral circulation, there is an exchange of mass between the ocean basins brought about through the inverse barometer effect¹³ due to changes in atmospheric pressure. These level changes (ζ) are quite well correlated within the Indian Ocean (Table 1). The ratio of the standard deviations

Table 1 Standard deviations of annual mean sea level and atmospheric pressure in the Indian Ocean for the period 1966-80

Port Group	$(\overline{\eta^2})^{1/2}$ (mm)	Atmospheric pressure station	$(\overline{\zeta^2})^{1/2}$ (mm)	Correlation coefficient between η and ζ
North-west Australia	48	Darwin	6	0.77
Western Australia	38	Perth	8	0.54
South Australia	33	Adelaide	12	0.93

 $\zeta = -P_{\rm a}/\rho g$ is the anomaly of mean sea level arising from the inverse barometer effect, η is the observed anomaly of mean sea level.

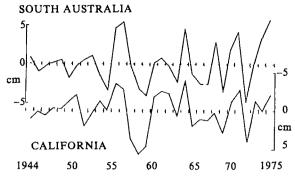


Fig. 2 Deviations in annual mean sea levels on the coasts of South Australia and California for the period 1944-75. Port groups: South Australia (Thevenard, Port Lincoln and Port Macdonnell (1959-75) and Port Adelaide (Outer Harbor 35°S, 138°E, 1944-70); California (San Francisco (35°N, 122°W), Los Angeles (34°N, 118°W), La Jolla (33°N, 117°W) and San Diego (33°N, 117°W)). The records for each California port²¹, and for the South Australian port group^{1,21} were detrended before the calculation of the deviations. Note the scale for the California port group is inverted.

 $(\overline{\zeta^2}/\overline{\eta^2})^{1/2}$ gradually increases from north to south, but even for South Australia, the inverse barometer effect accounts for only a small proportion of the variability in mean sea level.

Assuming that the anomaly of atmospheric pressure has the form, $P_{\bullet} = P_0 \sin \pi y / M$, and applying the geostrophic relation, $\rho_{\mathbf{z}} f u_{\mathbf{z}} = -\partial P_{\mathbf{z}} / \partial y$ in which $\rho_{\mathbf{z}}$ is the surface density of air, and $u_{\mathbf{z}}$ is the anomaly of the surface geostrophic wind, it can be easily shown that the anomalous difference in velocity between the trade winds and the westerlies is $|2\pi P_0/Mf\rho_a|$. Thus, substituting $P_0 = 1$ mbar (Table 1), $\rho_a = 1.2 \text{ kg m}^{-3}$, and $|f| = 10^{-4} \text{ s}^{-1}$, this velocity difference is 2 m s⁻¹, which, assuming a surface drag coefficient of 1.3×10^{-3} (ref. 14) is equivalent to an anomalous stress contrast between the trade winds and the westerlies of 0.06 N m⁻², in excellent agreement with the calculation based on the gyral circulation.

Note that the supra-annual behaviour of the atmospheric general circulation in the Indian Ocean was described in 1925 by Kidson¹⁵ using data for the period 1890-1923. Kidson selected a period of 36 months which he called 'a natural period of action and reaction' as being representative of the major variability. This period is somewhat less than periods (36-60 months) usually adduced from Pacific Ocean data in later studies¹⁶. The two data sets, however, may be reconciled through the discussion of the interaction between the two oceans given here.

We now present new data on tropical stratospheric geopotential heights¹⁷. The zonal annual mean anomalies for 10°N, 20°N and 30°N are all greater in magnitude than the surface pressure anomalies (Table 1), and are well correlated with the mean sea-level data (Table 2). The magnitudes of the correlation coefficients are higher with the South Australian than with the California mean sea-level data, the former being negative and the latter positive. It seems therefore that the zonal mean stratospheric pressure anomalies are in phase with the tropospheric pressure anomalies in the Indian Ocean, rather than linked barotropically with the Pacific Ocean pressure anomalies.

Table 2 Standard deviations of zonal annual mean geopotential heights at 50 mbar, and 10°N, 20°N and 30°N, for the period 1958-75, and the annual mean sea level for California and South Australian ports

		Mean sea	lev e l		
Latitude (°N)	Geopotential height $(p^2)^{1/2}$ (gpm)	California ports $(\eta^2)^{1/2}$ (mm)	South Australian ports $(\eta'^2)^{1/2}$ (mm)	coef	relation ficients tween p and n'
10 · 20 30	18 17 15	24	32	0.33 0.43 0.48	-0 55 -0 58 -0.64

An increase of 1 gpm at the sea surface would lower sea level, by the inverse barometer effect, 1 mm.

This remarkable observation is consistent with an earlier conclusion that the changes in the atmospheric general circulation would be more significant in the Indian Ocean than in the Pacific Ocean. Thus the Indian Ocean climatic signature would be more likely to be impressed on the zonal stratospheric data. Gordon¹⁸ has already reported a highly significant period of about 37 months from a spectral analysis of the monthly anomalies of the stratospheric data, suggestive of Kidson's conclusions for the surface Indian Ocean data. The period possesses greatest power between longitudes 120° and 180°E. Joseph¹⁹ has also found an approximate triennial oscillation in the wind at 100 mbar in the equatorial regions of Asia and Africa. In the peak westerly phase of the oscillation the subtropical westerly wind belt of the Northern Hemisphere penetrated southwards as far as or even beyond the Equator. He found that these incursions appeared to coincide with the failure of the Indian summer monsoon.

We have discussed evidence for a coupled long period interhemispheric oscillation of the ocean and the atmospheric, but the fundamental question is what maintains the oscillation. It may be argued that the ocean is simply responding to the atmospheric southern oscillation which has a similar variability to that deduced from the oceanographic data²⁰. We suggest, however, that the oceanic anomalies are induced by the variability of the monsoonally generated oceanic heat flux, the effect of which is to modulate the vorticity balance of the sub-tropical oceanic gyres. This argument has the important corollary that the subtropical atmospheric circulation must also change sympathetically to support the changes of ocean vorticity.

Thus our proposed mechanism implies that the ocean is the active partner in the supra-annual variability discussed which is characterized in the ocean by an interhemispheric oscillation, and in the atmosphere by the southern oscillation.

We thank Professor G. W. Lennon for use of the Mean Sea Level Data Bank, Dr J. S. Godfrey for early discussions on mean sea levels around Australia, and Dr R. A. D. Byron-Scott for comments. The stratospheric data set was provided by Professor K. Labitzke.

Received 24 September 1981; accepted 7 January 1982

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Phanerozoic land-sea and albedo variations as climate controls

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It has been suggested 1-8 that variations in the latitudinal distribution of land and sea have been important in controlling climate, especially the initiation of glacial epochs, through geological time. It is thought that surface albedo may be increased and ice caps initiated by the placement of land (and hence snow cover) near the poles^{2,6} or by the placement of land in low latitudes^{4,5,7} or the placement of land with extensive deserts in low latitudes⁸. I document here the relevent Phanerozoic land-sea variations based on recent global palaeogeographical reconstructions⁹, to assess total surface albedo variation and use these data to test the above mentioned hypotheses.

Palaeocontinental reconstructions for the Cambrian to the Triassic (570-200 Myr) have been attempted using all reliable palaeomagnetic data available in May 1981. These maps (Fig. 1) were constructed using the smallest subdivisions of geological time allowed by the data (epochs, early, medial, late Cambrian and so on). As usual with such palaeomagnetically defined reconstructions, longitudinal placement is often uncertain (especially during the early Palaeozoic) whilst latitudinal position is usually constrained to within ±10°, though for several blocks the precision may be much better. Fortunately, palaeoclimatic hypotheses are based almost solely on palaeolatitude. The palaeolatitudinal positions of certain continental blocks (North and South China, South-East Asia, Kolymia¹⁰) are uncertain for much of the Phanerozoic and their positions have been plotted by interpolating between and extrapolating beyond the currently available palaeomagnetic data. The distribution of

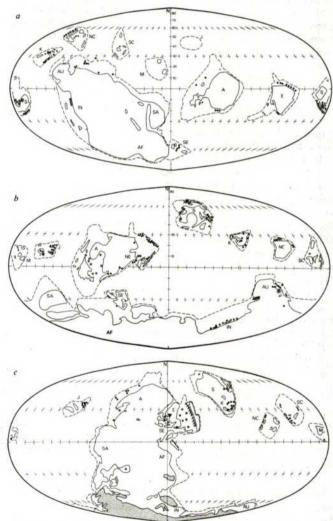


Fig. 1 Palaeogeographical maps for: a, late Cambrian; b, early Devonian; c, early Permian. Solid lines indicate palaeoshorelines; ●, major volcanics; ★, evaporites; ×, coral reefs. Major ice-caps are shown by stipple. The plot is a combined Lambert cylindrical and sinusoidal equal-area projection. The extent of continental blocks is defined elsewhere¹¹⁰. A, North American block (including Greenland); AF, Africa; AU, Australia; IN, India (including Tibet); J, Jano-Kolymian block; K, Kazakhstan block; M, South-East Asian block; N, Palaeo North Pole; NC, North China block; NE, Northern European block; S, Siberian block; SC, South China block; SE, Southern European block.

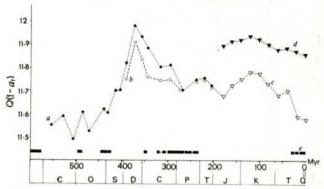


Fig. 2 Variation of $Q(1-a_{\rm T})$ (solar radiation absorption) at Earth's surface through the Phanerozoic. See ref. 8 for details of calculation. a, \blacksquare , $Q(1-a_{\rm T})$ values assuming no deserts or, for Permo-Trias, only those areas known from geological evidence to have been desert from the geological record; b, \bigcirc , values with desert prescribed $10^{\circ}-30^{\circ}$; c, \triangle , post-Triassic trend from ref. 8 assuming no deserts; d, \triangle , post-Triassic trend from Barron et al.8 assuming deserts $10^{\circ}-30^{\circ}$; e, major Phanerozoic ice ages.

Palaeozoic and Triassic epicontinental seas was then plotted using over 3,000 recent references.

The distribution of epicontinental seas is critical in consideration of past albedo as these seas have often almost completely inundated certain large continental blocks and they cannot be ignored as was done by $Cogley^{7.11}$. Palaeogeographical atlases 12,13 based on outmoded data and preconceptions, although still used by some $^{8.14,15}$ have not been used here. A preliminary approximation of major land surface types (deserts, forests and so on) has also been attempted mainly from a synthesis of the sedimentological and palaeobotanical literature. Thus the Cambrian-Triassic surface albedo variations shown in Fig. 2 should be as accurate as is possible from the current literature. To ensure comparability with the work of Barron et al. 8 a plot of $Q(1-a_T)$ is given (Fig. 2) rather than albedo, where Q is the total solar radiation received over the total area of a latitudinal belt and a_T is the surface albedo. Thus $Q(1-a_T)$ is the amount of absorbed radiation at the surface of the Earth.

Values of albedo for various surface types and sea at different latitudes are those given in Barron $et\,al$. Snow is not prescribed as there is little geological evidence for such cover. Prescription of snow cover between 60° and 90° for the early Carboniferous decreases the $Q(1-a_T)$ from 11.883 to 11.771. All land is prescribed a desert albedo before the Devonian. The first land plants are probably late Silurian and although lichens may have covered the early Palaeozoic land surface there is no evidence for this and a desert landscape is considered more probable. The advent of abundant land plants during the Devonian decreased the surface albedo (increased $Q(1-a_T)$) appreciably (Fig. 2).

Thus the early Palaeozoic was a time of high surface albedo with $Q(1-a_{\rm T})$ values similar to or lower than those of today (Fig. 3). However, major early Palaeozoic glacial periods are known only in the late. Ordovician-medial Silurian of Africa and South America. Probable early Ordovician glaciation in Argentina is localized and there are no records of a medial Ordovician glaciation. Geographically restricted ice caps were probably present in South America and, perhaps locally, in Africa 17-19,20. A surprisingly high $Q(1-a_{\rm T})$ value is evident during the Devonian reducing to slightly lower values during the Carboniferous. However there is no obvious correlation between high surface albedo (low $Q(1-a_{\rm T})$) values and the onset of the major glacial periods during the Ordovician, Carboniferous and Tertiary.

If the latitudinal distribution of the land and sea is plotted (Fig. 3) for epochs preceeding ice-age initiation then no obvious correlation is found.

A low latitude terrestrial aggregation occurred in the late Cambrian (Fig. 3c) and certainly reduced $Q(1-a_T)$ (Fig. 2).

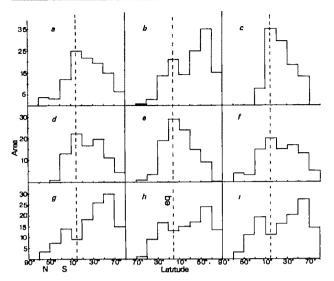


Fig. 3 Histograms of land area with respect to 20° palaeolatitude increments. Area $\times 10^6$ km². eq. Equator; a, early Cambrian; b, medial Cambrian; c, late Cambrian; d, early Ordovician; e, medial Ordovician; f, late Ordovician; g, early Carboniferous; h, medial Carboniferous; i, late Carboniferous.

However, no major ice cap was formed during the late Cambrian or early Ordovician although this may be due to a lack of high latitude land (Fig. 3c,d). Another near-equatorial aggregation of land occurred during the medial Ordovician (Fig. 3c), lowering the $Q(1-a_T)$ value and was succeeded by the major late Ordovician ice age at high latitudes. Thus the Cambro-Ordovician data suggest that ice-cap initiation requires both high albedos, generated by deserts at low latitudes, and sufficient land at high latitudes for ice to accumulate. An increase in absorbed radiation in the mid-Silurian (Fig. 2) may have been sufficient to end the ice-age. Unfortunately, such a scenario cannot be applied to the initiation of the late Carboniferous-Permian glaciation. Low surface albedo values preceded the glacial event during the Devonian and early Carboniferous mainly due to a relative dearth of low latitude land (Fig. 3). Similarly no obviously special configuration of land preceded the initiation of the southern ice-cap during the Oligocene^{8,21}. Thus the data presented in Figs 2-4 show that no obviously simple correlation exists between the latitudinal distribution of land and total surface albedo for epochs immediately preceding the initiation of ice-caps and a more complex explanation involving cloudcover albedo and interactions with other major climate determinations may have to be developed.

I thank C. Eastoe and B. Stait for invaluable help.

Received 9 June; accepted 3 December 1981

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Climatic conditions deduced from a 150-kyr oxygen isotope-pollen record from the Arabian Sea

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The correlation reported here of oxygen-18 and pollen records from off-shore marine cores1 enables the history of the nearby continental vegetation, which is controlled by climate, to be examined within the frame of the standard oxygen isotope stratigraphy. Core MD 76 135 from the northern Arabian Sea recovered sediments ranging from oxygen isotope stage 1, Holocene, to stage 6 before 128 kyr BP. Compared with the distribution of modern pollen, low sea-level glacial intervals contain well-preserved pollen which indicate saline littoral, arid and steppe inland conditions. High sea-level interglacial intervals are characterized by savanna-type vegetation. Pollens which are climatically diagnostic and wind-transported indicate distant source areas and reflect the intensification of low-level opposite air flows: the summer south-west monsoonal flow during the last interglacial and the beginning of the present interglacial, and the winter north-east trade winds during the last glacial maximum.

In present climatic conditions, the monsoon system dominates the atmospheric circulation over the Arabian Sea with seasonal low-level wind reversal following the latitudinal migration of the Intertropical Convergence Zone. In winter (October-May), steady but not very strong, dry north-east trade winds blow from Asia (Fig. 1). In summer (May-October), the cool, moist, steady and stronger southwesterlies, which are fed by the Southern Hemisphere winter south-east trade winds^{2,3}, bring heavy monsoonal rainfall to the south and east Asiatic continent

These low-level winds transport pollen from the continents to the ocean where it is eventually deposited in deep-sea sediments. Interpretation of pollen spectra in the Arabian Sea sediments relies on the present-day seasonal distribution of pollen in the air over the Arabian Sea, which identifies both its geographical source area and the wind responsible for its transport. We have studied this present-day distribution by collecting pollen with air filters on board ships travelling along the coasts^{6,7}. The cotton gauze filters are impregnated with silicon oil which collects and retains the mineral particles and pollen grains. They are renewed as often as necessary according to the nearby continental vegetation zones. In Table 1 we summarize data from the coast of Somalia during the summer monsoon and the winter trades. The pollen percentages refer to the total pollen.

Along the coast of Somalia, a 10-day record in July-August shows that the low-level south-west wind carries the highest percentages of Cyperaceae, Euphorbiaceae, Podocarpus and Olea. These taxa are derived from East Africa where the rainfall follows the migration of the Intertropical Convergence Zone of the tropical easterlies, and from the Ethiopian Highlands where rainfall originates in the African monsoon from the Atlantic Ocean⁸. They are well-dispersed East African pollen types^{9,10}.

Along the coast of Arabia, a 7-day record in November yielded mostly Chenopodiaceae and Artemisia pollen grains. The north and north-east low-level air flow suggests an origin in the Arabian desert and the Beluch and Iranian plateaux. The Thar desert does not appear as a source area, as in its southern

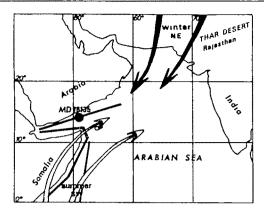


Fig. 1 Location of core MD76135. Arrows indicate low-level seasonal wind flow. Lines indicate ship-routes with pollen collection on air-filters. Solid line: winter air-filters (cruise Orgon IV of R/V Jean Charcot in November 1977, cruise Osiris IV of M/S Marion Dufresne in November 1979). Double lines: summer air-filters (cruise Osiris IV of M/S Marion Dufresne in July-August 1979).

part, the pollen rain does not include large amounts of these taxa¹¹.

As already observed in the Sahara¹², aeolian pollen transport is effective over very long distances, and its influence is dominant in arid zones. There, ground dust and pollen are resuspended in the atmosphere by diurnal thermal turbulence, uplifted to 2,000 m or more, and then transported by strong (but not necessarily prevailing) winds¹³. Over the Atlantic, dust of Sahara desert origin has been estimated¹⁴ at 260×10⁶ tons per year. There is no estimate of the amount of dust from Somali, Arabian and Thar deserts over the Arabian Sea, although the importance of wind in the transport of clay minerals and quartz to the Arabian Sea bottom is recognized¹⁵. The high frequency of haze (solid aerosol) over the north-west Arabian Sea in summer16 indicates mobilization by thermal turbulence, and the low-level steady strong south-west flow¹⁷ suggests a Somali origin. However, occasional north-west storms from Arabia⁴ probably contribute to the haze. In winter, the haze disappears¹⁶. The steady, weak, low-level winds from the north, north-east and north-west would transport quartz dust from the Thar and Arabian deserts into the Arabian Sea18. The low-level air flow probably picks up a great deal of pollen from the littoral zone. Accumulated over several seasons in the inland deserts, dust and pollen would be uplifted to higher altitudes and then, feeding the low-level flow, settle progressively on the sea surface.

This preliminary present-day aeolian pollen survey, which is the means to interpret the climatic variations from the pollen record in Arabian Sea sediments, shows that the two conspicuously opposite climatic and wind-regime patterns are traceable by pollen: pollen collected in summer off Somalia indicates a south-west origin, and in winter off Arabia, a north, north-east and north-west origin.

To document the response of this monsoonal system to the changing boundary conditions during the late Quaternary, we studied core MD76135, collected at 14° 26'6" N, 50° 31'3" E, 1,895 m water depth (Fig. 1). We have measured the 18 O/ 16 O ratio of the planktonic foraminiferal species Globigerinoides ruber and the benthic foraminiferal species Cibicides wuellerstorfi. Analyses were performed using a Micromass 602C mass spectrometer and an experimental procedure described elsewhere 19 . Results are expressed as δ^{18} O defined by the relationship:

$$\delta^{18}O = \left[\frac{(^{18}O/^{16}O) \text{ sample}}{(^{18}O/^{16}O) \text{ PDB})} - 1\right] \times 1,000$$

where PDB is the Chicago PDB-1 standard. No correction has been applied to take account of *Cibicides* departure from isotopic equilibrium²⁰ as this single benthic species has been analysed continuously. Twenty-nine samples were routinely

treated for pollen analysis²¹ and yielded an average of 250 pollens each.

The isotopic records of the planktonic and benthic foraminifera show, as usual, several oscillations. These δ^{18} O variations closely match those of other cores from the Atlantic²², Pacific and Indian Oceans²³, in agreement with the hypothesis that the major signal in these records reflects isotopic changes in the ocean water mass resulting from the waxing and waning of continental ice sheets²⁴.

Following Emiliani²², interglacial stages are identified by odd integers increasing with age and glacial stages are identified by even integers also increasing with age. Isotopic stage 2 corresponds to the last glacial maximum centred about 18 kyr ago, whereas stage 5 represents the last interglacial as observed in equatorial areas²². Stage 5 has been divided into five substages, of which the oldest, 5e, correlates with the Eemian interglacial of north-west Europe²⁵⁻²⁷. Isotopic substage 5e is poorly represented in core MD76135: while glacial isotopic stage 6 is well represented, there is a hiatus corresponding to the transition from stage 6 to interglacial substage 5e, and the lower part of the substage 5e. The upper part of substage 5e is represented by 10 cm of sediment. The sedimentary record is continuous above that level (Fig. 2).

Core MD76135 has provided a well documented pollen diagram. The relative abundance of pollen taxa is shown in Fig. 2. Dominant taxa include Chenopodiaceae which are arid and halophilous and can have a double source area, either inland desert²⁸ or littoral sebkhas²⁹, the dominant source in our marine record. Gramineae and Cyperaceae reflect the environment of the Sudanese savanna. These three dominant taxa originate in the near-shore and inland vegetation. The pollen diagram also contains various less abundant taxa which are presented in Table 2 according to their phyto-geographical source area and their ecology: humid tropical; from open woodland to mangrove, tropical montane (Olea, Podocarpus), from East Africa, Ethiopia and Yemen, Sudano-Sahelian savanna in Somalia, Sahelian near-shore steppe in Arabia and Somalia, Mediterranean subarid steppe (mostly Artemisia) from the Middle East plateaux. All the taxa are still extant in the area.

Figure 2 (right-hand side) shows variations in the pollen groups most diagnostic of climate and wind-regime (Table 2, first five columns): East African total humid tropical taxa carried by south-west winds, dry tropical Sahelian taxa from near-shore steppe, Mediterranean steppe taxa from the north-east. The three dominant taxa of the diagram are excluded (Gramineae, Cyperaceae, Chenopodiaceae). Thus the diagnostic taxa are expressed as percentages of a partial sum which represents only ~20% of the total pollen sum.

The comparison of the pollen and isotopic records of core MD76135 shows that interglacial substage 5e is marked by high relative frequencies of the Sudanese savanna and humid tropical pollen taxa which include the long distance transported tropical African Montane *Podocarpus* and *Olea* signalling a strong south-west monsoonal air flow, while Chenopodiaceae, Sahelian steppe and Mediterranean steppe *Artemisia* pollen decrease. We interpret this period to be both warm and humid. The lowest part of post-glacial isotopic stage 1, early Holocene, around 8 kyr BP at 130 cm core depth, is also characterized by

Table 1 Pollen data collected from the coast of Somalia

		Arabia	Som	alia
	Month	November	November	July-August
L	atitudes	11°5,N-16°4 N	1°0 N-10°3 N	11° N-0°3 N
T	otal pollen	1,363	701	1,681
9	6 Chenopodiaceae	59.2	18.6	13.2
9	6 Artemisia	24.9	3.1	0
9	6 Cyperaceae	0	0.4	3.3
9	6 Podocarpus			
	+ Olea	0	0.1	1.9
%	Euphorbiaceae	0.1	1.7	3.9
9	6 Gramineae	6.5	38.6	54.1
9	6 Others	9.3	37.5	23.6

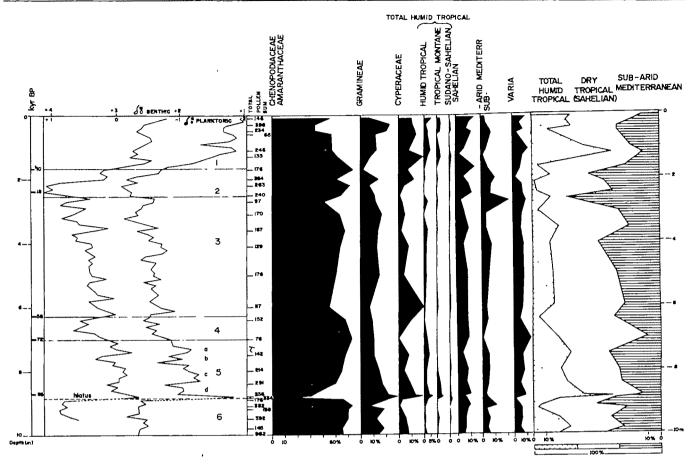


Fig. 2 Oxygen isotope and pollen record of core MD76135. Ages are estimated from refs 24, 41, 46. On the right column, total humid tropical taxa include humid tropical, tropical montane and Sudano-Sahelian taxa of the main diagram. All pollen relative frequencies are calculated on the total pollen sum.

highly increased amounts of humid tropical and Sudano-Sahelian savanna taxa, indicating a stronger south-west monsoonal flow, an increased Indian monsoon, and more moisture in Somalia. The late glacial-early Holocene (Bølling-Alleröd to Boreal) was the most humid period since the last glacial maximum in all the equatorial latitudes of the planet³⁰, a response to the insolation peak in summer centred at 11 kyr BP, controlled by the variations of the Earth's orbital parameters³¹. In East Africa, this humid phase, clearly displayed by high lake levels and pollen records^{30,32-40}, begins at 12.5 kyr BP, and its peak is reached from 10 to 8 kyr BP. In our record, around 12.5 kyr BP, at 200 cm core depth immediately after the most arid last glacial

maximum at 220 cm core depth, there is a sign of locally increased moisture fostering the Somalia savanna, and of intensified long distance transport of humid tropical pollen. The same level of moisture and wind intensity is found during isotopic stage 3, while during isotopic stage 5, it is always significantly higher. Between the incipient humidification (around 12.5 kyr BP) and the Holocene main humid peak, the cold/dry Younger Dryas (11–10 kyr BP), clearly marked in Fig. 2 by the step of the isotopic curve at the transition of stages 2 to 1 (ref. 41), at 165-m core depth, correlates with local desiccation (decline of Gramineae-Cyperaceae, rise of Chenopodiaceae and Sahelian), and weakening of the monsoonal wind (decline of

Humid tropical	East African total humid t Tropical montane	ropical Sudano–Sahelian	Dry tropical sahelian	Sub-arid Mediterranean steppe	Varia
Alchornea Apocynaceae Avicennia Combretaceae Meliaceae Rhizophora Sida Spores	Olea Podocarpus	Acanthaceae Capparidaceae Celtis Dobera glabra Hypoestes	Abutilon Acacia Blepharis Caesalpinia Cassia Commiphora Compositae tub. Cruciferae Heliotropium Hyphaene Indigofera Maerua Phyllanthus Salvadora persica Tribulus Ziziphus	Artemisia Calligonum Centaurea Compositae lig. Ephedra Erodium Ombelliferae Plantago	Anacardiaceae Betula Caryophyllaceae Cleome Convolvulus Cordia Corylus Euphorbia Fagonia Fagus Labiateae Myrica Nitraria Papilionaceae Plumbaginaceae Pinus Rhus

humid tropical). This is also widely displayed in Africa³⁰. In the western Arabian Sea, early Holocene faunal planktonic assemblages point to lower sea-surface temperatures and stronger south-west monsoon air flow⁴². Today, the summer south-west air flow induces strong upwelling along the Somalia and Arabia coasts, which is responsible for the extensive seasonal cooling of the Arabian Sea⁴³. In Rajasthan, north-west India, lake levels and pollen records point to the most humid monsoonal period between 10.3 and 9.5 kyr BP (ref. 44) still as humid but with more winter rain of Mediterranean origin from 9.5 kyr BP, until 5.0 kyr BP (refs 44, 45).

Glacial isotopic stages 6, 4, end of 3 and most clearly stage 2, the last glacial maximum are marked by low relative frequencies of humid tropical taxa and high relative frequencies of taxa from the Mediterranean steppe. These latter taxa originate from the north and north-east, indicating stronger north-east trade winds and increased aridity. High sea-surface temperatures estimated from faunal analysis in the western Arabian Sea during the last glacial maximum reflect reduced oceanic upwelling, as the south-west air flow was weaker42

The arid halophilous Chenopodiaceae are abundant throughout the core and reflect mostly littoral vegetation²⁹. They decrease during interglacial isotopic substage 5e (together with the Sahelian steppe) and the upper part of stage 1 (late Holocene), when the sea level was high. However, in the Holocene, the most humid period as indicated by the highest relative frequency of savanna and humid tropical taxa, occurred at 130 cm core depth, before the Chenopodiaceae minimum at 50 cm core depth. During substages 5e to 5a. Chenopodiaceae increase while humid tropical taxa decrease. Glacial stages 6, 4 and 2, and also the transition from stage 2 to 1, Younger Dryas⁴¹, are characterized by high Chenopodiaceae and Sahelian steppe and low humid tropical taxa values, pointing to conditions drier than those of today. It is suggested that Chenopodiaceae reflect the salinity increase of the littoral water-table during glacial periods²⁹. Aridity lowers the freshwater table level in the newly uncovered, lower, regressive littoral strip. The seawater, which is then more saline, impinges for hydrostatic readjustment. Extensive littoral sebkhas vegetated by Chenopodiaceae result. This accounts for the very good positive correlation of Chenopodiaceae with the δ^{18} O enrichment and explains why the Chenopodiaceae Holocene minimum (maximum transgression) occurs significantly later than the Holocene most humid period.

We thus conclude that this marine pollen diagram from the arid tropical zone depicts the fluctuations of the intensity of the seasonally reversing low latitude wind systems, and of the local moisture, and correlates littoral aridity with sea-level variations. These fluctuations correlate with the global climate changes as indicated by isotopic stratigraphy. The intensity of the monsoonal winds seems to respond mainly to the variations of the insolation, controlled by the Earth orbital parameters, being maximum when the insolation is strongest⁴⁶. Glacial periods were arid in southwestern Asia and at low latitudes, and the north-east trade winds were intensified against a decreased monsoonal flow. In the tropics, as evidenced by the most detailed Holocene record, incipient interglacial stages were the most humid periods: the south-west monsoonal flow was intensified, over the Atlantic and Africa, and over the Arabian Sea and India. The insolation peak developed low pressures over the tropical continents and heavy rains resulted. Meanwhile, the middle and high latitudes, more influenced by the northern ice-sheets, also had warm summers⁴⁷ although dry, and reached their humidity peak later.

We thank J. Antignac and B. Le Coat for help in the isotopic analyses, D. Duzer for technical assistance in the air-filters pollen analyses, W. Prell for a careful review and useful comments. Cruises Osiris II and Osiris IV of the M/S Marion-Dufresne have been supported by TAAF. Cruise Orgon of the R/V Jean Charcot has been supported by CNEXO. Support for laboratory studies was provided by CNRS, DGRST and CEA. (All of us are supported by CNRS.)

Received 21 September, accepted 22 December 1981.

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A-nor-steranes, a novel class of sedimentary hydrocarbons

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As part of our investigations of Cretaceous black shales¹ we have analysed samples from a location near Aqualagna in the north central Apennines, Italy (43°39' N, 12°43' E). The saturated hydrocarbon fraction of one sample, a dark-grey shale of Upper Barremian age (115 Myr BP), contains novel series of steranes, which have been identified as C_{26} – C_{28} 5β (H)- and $5\alpha(H)$ -A-nor-steranes. Because these compounds are structurally related to 3β -hydroxymethyl-A-nor-steranes which have been found in some sponges, we suggest here that these steranes might be biological markers for certain types of sponges.

The Upper Barremian sample has an organic carbon content of 9% and was Soxhlet extracted with toluene/methanol (1/3,

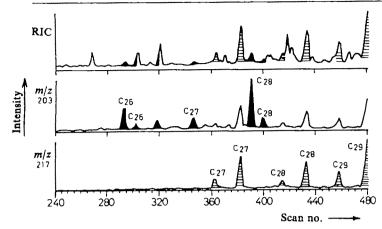


Fig. 1 Part of the reconstructed ion chromatogram (RIC) and of mass fragmentograms of m/z 203 (indicative of A-nor-steranes) and m/z 217 (indicative of steranes). Temperature programme: $150-280 \,^{\circ}\text{C}(4 \,^{\circ}\text{C min}^{-1})$.

v/v; 20 h). The hexane-soluble part of the extract was separated by column chromatography over silica/alumina using hexane, toluene and toluene/methanol (1/1, v/v). The saturated hydrocarbons were isolated from the hexane fraction by Ag⁺-TLC (Merck silicagel impregnated with a 3% solution of silver nitrate in water/methanol) using pentane as a developer. Gas chromatography-mass spectrometry was performed on a Varian 3700 gas chromatograph equipped with a 25-m glass capillary column (i.d. 0.25 mm) coated with CP-sil-5 coupled to a MAT 44 quadrupole-mass spectrometer operating at 70 eV (mass range: m/z 50-500; cycle time, 2 s).

The saturated hydrocarbon fraction of the shale sample contains series of n- and isoprenoid alkanes, steranes, 4-methylsteranes and hopanes. Also present are a series of compounds with mass spectra that show a main fragment ion at m/z 203 (Fig. 1) and molecular ions at m/z 358 (Fig. 2a), 372 and 386. These mass spectra are similar to those of steranes with m/z 217, 218 shifted to m/z 203, 204 respectively and m/z 149 shifted to m/z 135.

Based on the mass spectra we assumed the unknown compounds to be a series of modified C_{26} , C_{27} and C_{28} steranes with 14 mass units missing from the nucleus and with side chains comparable to the common C_{27} , C_{28} and C_{29} steranes. From the shift of the fragment ion m/z 217—in steranes representing the ABC-ring part of the molecule²—to m/z 203 and the shift of the fragment ion m/z 149—in steranes ascribed to an AB-ring fragment³—to m/z 135 it can be deduced that a methylene group from ring A or B or the C_{19} methyl group is not present in these compounds. The presence of a characteristic peak at m/z 262 in the spectrum of the C_{26} unknown compound suggests that the missing carbon atom is the 19-methyl group or a methylene group of ring A. Ion m/z 262 in the spectrum of cholestane results from B-ring cleavage⁴ and therefore a substantial change

in the intensity of m/z 262 should be expected in the case of a B-nor-sterane.

To test our hypothesis the following compounds were synthesized⁵⁻⁹ (conversion of 19-hydroxycholest-4-en-3-one into 19-nor-cholesterol was achieved according to a reaction scheme obtained from Organon) (Fig. 3): 19-nor-cholestane (the synthesis yielded three compounds with identical mass spectra, probably A-B ring junction isomers), $5\alpha(H)$ -A-nor-cholestane and $5\beta(H)$ -A-nor-cholestane. The mass spectra of the 19-nor-cholestanes differed from those of the A-nor-cholestanes in the presence of a prominent peak at m/z 148 (Fig. 2d). The mass spectra of the $5\alpha(H)$ - and $5\beta(H)$ -isomers of A-nor-cholestane are different with regard to the ratios of M⁺ and M⁺-15 (Fig. 2b, c).

Mass spectrometry (Fig. 2) and coinjection on a capillary column coated with SE-52 showed that the first eluting C_{26} -unknown compound (Fig. 1) is identical to 5β (H)-A-nor-cholestane. The second unknown C_{26} -compound coeluted with 5α (H)-A-nor-cholestane but the mass spectrum of this less abundant unknown was not intense enough to compare it in detail with the spectrum of the synthetic compound. Therefore the second eluting C_{26} -compound is tentatively identified as 5α (H)-A-nor-cholestane. From these results it seems likely that the hydrocarbon fraction of the shale sample under investigation contains series of C_{26} , C_{27} and C_{28} , 5α (H)- and 5β (H)-A-nor-steranes.

In addition to these $5\alpha(H)$ - and $5\beta(H)$ -A-nor-steranes there is one other relatively intense peak in the fragmentogram of m/z 203 (Fig. 1, scan 318). The mass spectrum of this compound does indeed show an intense peak at m/z 203 but its identity is unknown at present. An assignment of this compound to a C_{20} -epimer of an A-nor-sterane seems very unlikely as the regular steranes in this sample are exclusively present in the natural 20R configuration.

It is now clear that the nor-steranes in DSDP sediments previously assigned tentatively as 19-nor-components¹⁰ are in fact A-nor-steranes (J. McEvoy, personal communication).

The unsaturated hydrocarbon fraction of the sample contains diasterenes, hopenes and in addition a series of compounds with mass spectra similar to those of diasterenes but with a base peak at m/z 243 instead of m/z 257 (ref. 11). Considering the presence of A-nor-steranes in the saturated hydrocarbon fraction we suggest that this series of unsaturated compounds is a series of A-nor-diasterenes. However, this suggestion has to be confirmed through the synthesis of the proper compounds.

As abiotic ring contraction of six-membered rings has not been reported in sediments, the presence of A-nor-steranes in the sample under investigation is thought to be the result of the input of related compounds showing this skeleton.

Sterols with a $5\alpha(H)$ -A-nor-nucleus and a 3β -hydroxymethyl group have been found as major sterols in several sponges $^{12-14}$. With one exception these sponges all belong to the family Axinellidae 14 . It is not known, however, whether these sponges already existed during the Cretaceous. The A-nor-sterols are

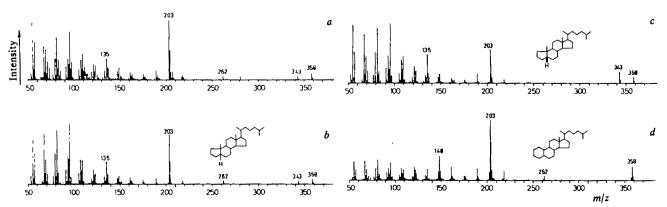


Fig. 2 Mass spectra of a, the first eluting unknown C₂₆-compound (scan 293, Fig. 1); b, synthetic 5β (H)-A-nor-cholestane; c, synthetic 5α (H)-A-nor-cholestane; d, synthetic 19-nor-cholestane.

Synthetic pathways for: a, 19-nor-cholestane from 19-hydroxy-cholest-4-en-3-one (refs 6-8); b, A-nor-cholestane ($5\alpha(H)$ and $5\beta(H)$) from cholestanone $(5\alpha(H))$ and $5\beta(H)$ (refs 6-9).

formed by the sponges through ring contraction of the dietary sterols¹⁵. It seems possible that the A-nor-steranes present in this Cretaceous sample are the ultimate diagenetic products of 3β -hydroxymethyl-A-nor-steranes, assuming that the 3β hydroxymethyl group is removed (through microbial action?) and that isomerization at C5 occurs at some stage of diagenesis resulting preferentially in the more stable $5\beta(H)$ -isomer.

Molecular mechanics calculations using the EAS force field¹⁶ by J. M. A. Baas (Delft University of Technology) showed that model compound I (A-B cis) is thermodynamically more stable than model compound II (A-B trans) by 1.9 kcal mol⁻¹ (Fig. 4). See also calculations on the 8-methyl-hydrindanes in ref. 17. The presence of sponges seems to contradict the supposedly anoxic environment of sedimentation of black shales. However, some sponges are tolerant of very low levels of oxygen¹ Moreover, black shale formation does not necessarily imply the existence of a thick anoxic water layer but can also occur when anoxic conditions are restricted to the sediment itself or to a thin (few millimetres) water layer at the bottom. We therefore suggest that these novel series of sedimentary hydrocarbons might be useful markers for certain types of sponges.

We thank P. L. de Boer for the sample and Dr F. J. Zeelen (Organon, Oss) for 19-hydroxycholestenone. This research is supported by the Netherlands Foundation for Earth Science Research (AWON) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO).

Received 23 October 1981, accepted 7 January 1982.

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Chemi-ionization in oxyacetylene flames

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The identity of the first ion formed in hydrocarbon flames has created much discussion. Two species have been favoured 1-7, produced in the chemi-ionization processes:

$$CH + O \rightarrow CHO^{+} + e^{-} \tag{1}$$

$$CH^{+} + C_{2}H_{2} \rightarrow C_{3}H_{3}^{+} + e^{-}$$
 (2)

Reaction (2) is usually taken to involve electronically excited CH, in which case reaction (2), like reaction (1) with ground state CH, is close to thermoneutral. Experimental evidence has been claimed for both. For example, Peeters et al.3 prefer reaction (1), while Calcote et al.4 have suggested that reaction (2) is necessary to explain the abundance of ions in fuel-rich flames. Interestingly, evidence for CHO+ has been obtained1.3.5 from either non-acetylene flames or those with low C/O ratios, while evidence for $C_3H_3^+$ appears 2,4,6,7 in fuel-rich acetylene flames, where [C₂H₂] is high and [O] is low. We describe here a mass spectrometric study of fuel-rich acetylene flames, and attempt to clarify the picture. Evidence is presented which suggests that both reactions operate simultaneously in oxyacetylene flames.

Experimental details have been given elsewhere⁸. Briefly, pre-mixed laminar fuel-rich acetylene/oxygen/argon flames were burnt at atmospheric pressure on a single quartz tube. We are concerned here with three such flames, all having similar velocities and temperatures (~2,300 K), but with C/O ratios of 0.80, 1.02 and 1.09. With C/O = 1.02 and 1.09 a 'feather', that is, an extended luminous mantle, is visible downstream of the main reaction zone. Soot is only produced in the flame with C/O=1.09. Ion concentrations were measured along each flame axis using a quadrupole mass spectrometer. Such concentration profiles could be obtained for single ions, or, by removing the d.c. component of the rod voltage, of the total ion current.

Various classes of positive ion emerge in this study. All hydrocarbon ions $C_x H_y^+$ reach peak concentration in the reaction zone or just downstream of it. In fact, they are of two types: aromatic ions, which decay extremely rapidly in the feather, and polyacetylene ions, which persist as far as the feather does and then decay quickly in the burnt gases.

However, major oxygen-containing ions, including H₃O⁺, CHO⁺ and C₂H₅OH₂⁺, have concentrations which peak in the burnt gases and then persist for several millimetres downstream. This is typified by the profiles for C₃H₃⁺ and CHO⁺ shown in Fig. 1. Figure 1a is for C/O = 0.80, where no feather is present. $C_3H_3^+$, which is the most abundant of the $C_xH_y^+$ species, reaches peak concentration in the reaction zone and disappears very rapidly in the burnt gases, while CHO+ peaks some 3 mm downstream of the reaction zone. As the fuel-oxygen mixture is enriched, the feather appears for C/O > 0.9; with C/O = 1.02(Fig. 1b) the feather is ~5 mm long. C₃H₃⁺ still peaks in the reaction zone (RZ) and is present throughout the feather, peaking again before decaying at the feather tip. However, CHO+ reaches maximum concentration downstream of the feather tip, and is not detected at all in the reaction zone. Soot is actually seen in the richest flame studied here (C/O = 1.09,Fig. 1c), which makes the length of the feather difficult to estimate. However, CHO+ peaks some 10 mm downstream of the reaction zone. Thus can an ion which is in such low concentrations and only seen in detectable quantities so far downstream, really be the only primary chemi-ion?

Figure 2, a plot of total positive ion current against distance, provides more information. All profiles are undoubtedly double-peaked, noticeable more as a shoulder for C/O = 0.8. The first peak is in the reaction zone, while the second appears just downstream of the feather tip. In fact, these two peaks correspond spatially to maxima for the ions $C_3H_3^+$ and CHO^+ .

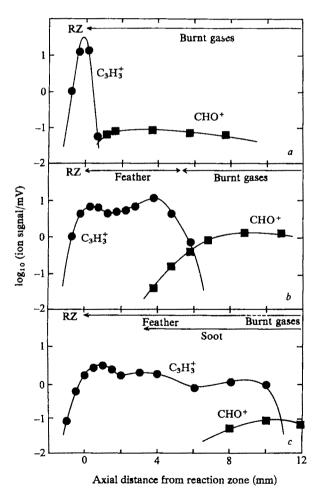


Fig. 1 Concentration profiles of $C_3H_3^+$ (\blacksquare) and CHO⁺ (\blacksquare) in acetylene/oxygen/argon flames plotted as \log_{10} (ion signal) against distance along flame axis. Zero distance corresponds to the primary reaction zone (RZ), as denoted by the emission of C_2 . Flames are: a, unburnt gas composition (by volume): $[C_2H_2]/[O_2]/[Ar] = 0.80/1.0/6.4$, so that C/O = 0.80; b, $[C_2H_2]/[O_2]/[Ar] = 1.02/1.0/5.3$, C/O = 1.02; c, $[C_2H_2]/[O_2]/[Ar] = 1.09/1.0/5.8$, C/O = 1.09.

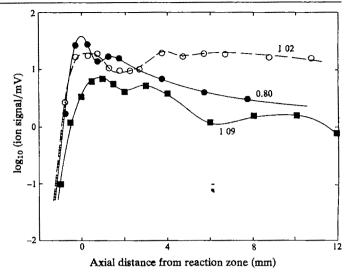


Fig. 2 Total positive ion intensity profiles in acetylene/oxygen/argon flames, with C/O ratios as shown, plotted as log₁₀ (ion signal) against axial distance.

The second increase in total ion current indicates a fresh production of ions by a different mechanism, rather than chemical production from existing hydrocarbon ions. The profile plotted in Fig. 2 for the sooting flame (C/O=1.09) is actually the sum of masses 0-170 AMU, rather than the total abundance of all positive ions. There is a distinction between these two quantities in this flame, which arises from the presence (but in sooty flames only) of positive ions having masses beyond the range of this instrument. The form of Fig. 2 avoids any complications from these heavy ions.

The two maxima in Fig. 2 and their relationship with the profiles of $C_3H_3^+$ and CHO^+ in Fig. 1 lead us to propose that two primary ionization mechanisms operate in fuel-rich acetylene flames, namely reactions (1) and (2). As the C/O ratio is increased, it is expected that the concentration of acetylene will grow, while oxygen atoms will decrease. Consequently, the rate of reaction (1) (C[CH][O]) will decrease, while that of reaction (2) ($C[CH^*][C_2H_2]$) will increase. The evidence above suggests that reaction (2) can become faster than reaction (1), although both are operating. The result is two separated zones of ion production. In lean acetylene flames and systems using other fuels, $[C_2H_2]$ will probably be low enough for reaction (1) to dominate. In fact, in very lean acetylene flames (CO = 0.06), very little $C_3H_3^+$ is observed at all⁹. With CHO⁺ as primary ion, hydrocarbon ions may be produced by processes such as:

$$CHO^{+} + CH_{2}O \rightarrow CH_{2}OH^{+} + CO$$

 $CH_{2}OH^{+} + C_{2}H_{2} \rightarrow C_{3}H_{3}^{+} + H_{2}O$

This may explain the second (more downstream) peak in the profile of the hydrocarbon ions.

These results confirm and expand the work of Tse et al.⁶, who reported that (1) CHO⁺ and H₃O⁺ peak concentrations were a long way downstream of C₃H₃⁺, and (2) hydrocarbon ions were pyrolytic in origin and preceded true oxidation. The chemistry of neutral radicals in the feather seems to have received little attention in the past, although much is known of stable products ^{10,11}. Ogden has made emission spectroscopic measurements directly on CH* and indirectly on H, OH and O; they indicate that [CH*] has a double-peaked profile which maximizes in the reaction zone and again early in the feather, while H, OH and O maximize in concentration just once, further downstream in the feather. However, as excitation of CH is chemical rather than thermal, no inferences should be made concerning ground state CH, which participates in reaction (1). Some quantitative data on this species would be useful in testing the viability of two chemi-ionization processes operating simultaneously. Even so, our conclusion that there

are two chemi-ionization processes agrees with what is known about the concentration profiles of the relevant neutral species. We thank the British Gas Corporation for financial assistance.

Received 26 November 1981; accepted 26 January 1982.

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Preservation of unsaturated fatty acids in Palaeogene angiosperm fruits and seeds

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Despite the rapidity with which unsaturated fatty acids (UFAs) undergo autoxidation by atmospheric oxygen. Priestlev et al. have reported that oleic and linoleic acids persist in Zea mays seeds >1,500 yr old. They suggest, on the basis of analyses of progressively older seed samples (78-1,700 yr old), that a small proportion of UFAs may persist in seed tissues over extended periods of time. Here we report chemical analyses of Eocene and Oligocene angiosperm fruits and seeds (30-50 × Myr) which indicate the preservation of significant amounts of unsaturated fatty acids (9.6-17.8% of the total lipid content) in these ancient organic remains. The fossils contain larger proportions of polar unsaturated fatty acids, derived primarily from membranes, than those derived from storage lipids, suggesting a preferential oxidation of unsaturated fatty acid constituents of the membranes. We suggest that morphological characters, such as thin seed coats and the accessibility of the inner seed structure to the environment, may influence the extent of unsaturated fatty acid preservation.

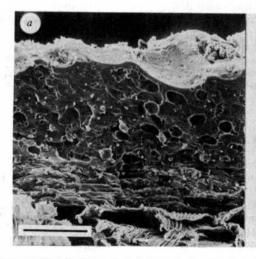
Fossil material was collected from two localities of different age (Table 1). Seeds of Zanthoxylum ornatum were obtained

from the Middle Eocene (~45 Myr old) Geiselthal Flora of East Germany2. Other fossils were collected from the Brandon Lignite of West-Central Vermont, the flora of which is now being investigated3,4. The age of the Brandon flora is uncertain, but is presently inferred to be Middle to Late Oligocene (30 Myr old). In both cases the fossils were found as compactions (threedimensional structures composed of original organic material) preserved in brown coal. These coals are the result of the compression of peat deposits in the swampy environments in or around which the parent plants grew. The fossils most probably were preserved in a waterlogged, anaerobic environment since the time of their deposition. The preservation is excellent in all cases, for example, seeds of Zanthoxylum rhabdospermum and Euodia costata retain the non-lignified secondary thickenings of the inner wall of the inner integument5. Four of the five fossils examined are seeds. Of these, Z. ornatum2, Z. rhabdospermum and Euodia lignita 5 possess sclerotic, semi-vitreous seed coats of 8-30 cell layers, 150-700 µm thick (Fig. 1a). In contrast, the seed coat of Vitis macrochalaza4 is thinner (1-2 cell layers, 60-225 µm thick), and is more leathery than vitreous^{6,7} Symplocos sp., in contrast to the other fossils examined, is a trilocular endocarp bearing remnants of thin-walled seeds. Each locule opens to the exterior through a large pore, exposing the inner as well as the outer face of the fossil to ground water.

Representative fossils were collected from moist lignite, stored in glass vials, and subsequently crushed to a fine powder; to prevent oxidation of potentially preserved UFAs, we added an antioxidant (2,6-di-tert-butyl-p-cresol; 50 µg ml-1). Extraction of fatty acids and hydrocarbons used the saponification procedure of Farrington and Quin8. Samples of the fossil material were refluxed (1 h) with 0.5 M KOH in 95% methanol/benzene (1:1 v/v). Non-saponifiable lipids were separated from saponifiable compounds by adding water to the saponification solvent mixture and extracting with petroleum ether. The aqueous phase of this separation was acidified and saponified lipids were extracted with petroleum ether. Fatty acids were isolated by methylating the dried residue of the saponifiable lipid extract using the BF3-MeOH procedure of Metcalfe et al.9. Polar lipids (phoso- and glycolipids) were separated from storage lipids using the analytical technique of Priestley et al.10 and Dittmer and Willis11. Fatty acid methyl esters were extracted with petroleum ether and purified by TLC on silica gel G plates. Identification of UFAs used combined gas and mass spectroscopy.

Microbial activity in the fossil specimens examined was evidenced by the detection of trace amounts of isomeric cisvaccenic acid (18: 1w7), which is an abundant bacterial constituent12. Mass spectroscopy, however, revealed that the most prevalent 18:2 acid in our samples was 18:1w9 oleic acid. From comparisons of microbially decomposed seed tissues of some of the fossil taxa found as fossils, and the geolipids isolated

Fig. 1 a, Scanning electron micrograph of the seed wall of a broken Z. rhabdospermum seed (Brandon, Vermont). The spirally-thickened cells of the inner integument (at bottom) are succeeded by those of the bilayered outer integument, the inner portion of which consists of approximately five levels of bricklike cells and the outer of four to five levels of isodiametric sclereids. Scale bar, 50 µm. b, Scanning electron micrograph of the base of a trilocular endocarp of Symplocos sp. (Brandon, Vermont). Note the three pores, each of which leads to a locule carrying the remains of a thin-walled seed. Scale bar, 1 µm.



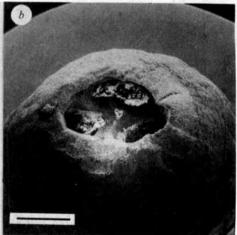


Table 1 Palaeobotanical data for fossil specimens examined

Specimen	Family	Locality	Age	Average wall thickness (μm)	No. of cell layers
Zanthoxylum ornatum Chandler	Rutaceae	Geiselthal, East Germany	Middle Eocene	300700	20-30+
Zanthoxylum rhabdospermum Tiffney	Rutaceae	Brandon Lignite, Vermont	Middle Oligocene?	200-400	8-20
Euodia costata Tiffney	Rutaceae	Brandon Lignite, Vermont	Middle Oligocene?	150-250	815
Symplocos sp	Symplocaceae	Brandon Lignite, Vermont	Middle Oligocene?	650-1,000	14-20
Vitis macrochalaza Tiffney	Vitaceae	Brandon Lignite, Vermont	Middle Oligocene?	60-225	1-2

Table 2 Representative UFA content of fruits/seeds of the Brandon Lignite

THE RESERVE THE PROPERTY OF TH							rated fatty acids†
				atty acids		Polar	Storage
Specimen	(mg per g seed)	16 0	18.0	18.1	18 · 2	subfraction	subfraction
Zanthoxylum ornatum	7 4* (0.5)	36 0* (0.1)	47.9 (0.1)	10.3 (0.2)	3.7 (0.5)	6.3	7.7
Zanthoxylum rhabdospermum	13.4 (0 3)	48.0 (0 5)	34 0 (0.2)	15.7 (0.3)	2.1 (0 3)	5.7	14 1
Euodia costata	109 (0.9)	35.2 (0)	43 2 (0)	9.6 (0)	4.8 (0)	5.5	8.9
Symplocos sp	5 6 (0 7)	39 7 (0)	49.7 (0)	9.6 (0)	_	1.3	8.3
Vltis macrochalaza	77 (05)	40 3 (0)	44 8 (0)	8.4 (0)	4.0 (0)	5.6	6.8

Fatty acid methyl esters were identified on (1) a 2 m × 1.5 mm glass column packed with 20% FFAP on 70-80 mesh crom W-AW-DMCS operated isothermally at 190 °C. and (2) a 2 m×1.5 mm glass column packed with 15% diethylene glycol succinate on Anakrom ABS 110/120 mesh, operated isothermally at 145 °C.

from the fossil specimens examined here and from their associated inorganic strata, we conclude that biotic contamination of our specimens was minimal. The geolipid (UFA) profiles presented are interpreted, therefore, as being representative of the endogenous biochemical profiles of the fossil taxa.

With the exception of Symplocos, the fossils contain significant percentages of linoleic (C_{18 2}) and oleic (C_{18 1}) acids (Table 2), indicating that complete oxidation of the UFA fraction has not occurred despite the age of the specimens. The lack of C_{18 2} in the endocarp of Symplocos may be ascribed to the thin-walled nature of the seeds and their continuity with the external environment through the locular pores (Fig. 1b). While the relative proportion of palmitic acid (C_{16 0}) is greater than that of C_{18 0} in Zanthoxylum seeds, the remaining specimens show a repeated pattern in the relative abundance of the four major acids detected $(C_{180} > C_{160} > C_{181} > C_{182})$. This differs from the relative proportion of these acids found in modern counterparts $(C_{182} > C_{18.1} > C_{180} > C_{160})$, and is interpreted as evidence for the enrichment of the C₁₈₀ acid component by the oxidation of C_{18 2} and C_{18 1} constituents. Consistent with this notion is the observation that Symplocos endocarps lack detectable C_{18 2} and yield the highest percentage of C_{18 0} (Table 2). A comparison of the proportion of fatty acids isolated from the polar lipids (predominantly of membrane origin in modern seeds) and from storage lipid subfractions (Table 2) provides evidence for the preferential degradation of membrane

Our study of the UFA content of these fossils indicates that a degradation of the membrane UFA subfraction parallels the absence of preserved membrane structure. Transmission electron microscopy of fossil specimens from the Brandon Lignite revealed little or no demonstrable protoplasmic details. The deterioration of membranes may well have resulted from lipid peroxidation and the consequent perturbation of the characteristic biomolecular (lipid-protein) membrane infrastructure. Electron microscopy has demonstrated that some membrane structure survives in ancient seeds and leaf tissues, indicating that sufficient membrane lipids may persist to maintain the bilayer configuration 13,14. Priestley et al. reported that the UFAs of the storage lipid and membrane lipid subfractions of maize seeds 1,700 yr old were equally susceptible to oxidation. The apparent preferential preservation of storage lipid UFAs in the fossil material examined here could reflect some differential distribution of naturally occurring antioxidants in the tissues. The ability of tocopherols, in low concentrations, to prevent lipid peroxidation is well documented15. Gaillard16 has reviewed

the roles of antioxidants in lipid metabolism. Note that tocopherols accumulate in plastids and that their synthesis and that of storage lipids in seeds may be separate, independent processes. It is noteworthy that plastids appear to be the most persistent organelles in fossil leaf tissues^{13,14}.

The preservation of such labile components in fossil reproductive structures >50 Myr old, confirms the previous speculation1 that a residual, long-lasting UFA component will survive in old seed tissues. It is also possible that other labile constituents of chemotaxonomic significance may be preserved in reproductive organs such as seeds and fruits from the localities examined. Geochemical analyses of other fossil specimens have revealed a vast array of biochemical constituents commonly thought to be too labile to survive over extended geological time¹⁷. The data presented here can be placed in the context of reports documenting the preservation of flavonoids18, chlorophyll derivatives¹⁹ and various phenolics²⁰ in comparably old fossils and fossil strata. Further chemical and ultrastructural analyses of Brandon Lignite fossils and specimens from other localities may elucidate the biochemical processes involved in fossilization as well as provide information relevant to the taxonomy of problematical angiosperm genera.

We thank Drs D. H. Mai and H.-J. Gregor for providing specimens of fossil Zanthoxylum. This research was supported by a NSF grant to J.N.

Received 1 October 1981; accepted 12 January 1982

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^{*} Mean, range is shown in parentheses.

[†] Distribution of % total 18:1 and 18.2 acids in polar and storage lipid subfractions

Vision has a role in *Limulus* mating behaviour

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In 1928 Hartline noted that the lateral eye of the horseshoe crab, Limulus polyphemus, was an admirable preparation for research in visual physiology. Since that time, extensive studies of Limulus have yielded fundamental understandings of visual processes and of sensory processes in general1.2. However, except for demonstrations of primitive phototaxis3-5, studies have not yet revealed a possible role of vision in the animal's behaviour. We have further investigated the problem by studying mating behaviour, the only known behaviour exhibited by Limulus in its natural habitat. We report here that horseshoe crabs discriminate form and contrast during mating: males are attracted to painted cement castings of the female carapace and other forms, the degree of attraction depending on the form and contrast of the castings, and on the time of day. The discrimination of form may result from information transmitted by several sensory systems but the discrimination of contrast requires vision.

Along the eastern coast of North America, *Limuli* move in from deep water in the spring and build nests on protected beaches near the water's edge at high tide⁶. Numerous femalemale pairs arrive, together with an excess of males. We observed the activity of males in the vicinity of cement castings placed in the water at a nesting beach.

The cement castings were made from an adult female carapace (27 cm wide), a hemisphere (29.5 cm diameter) and a cube (1.640 cm²). The exposed surface areas of the hemisphere and cube were each equivalent to that of the female carapace (1,365 cm²). Each casting was painted black, grey or white, yielding relative contrasts against sand underwater of -0.70, +0.1 and +0.75, respectively. The average relative contrast of the Limulus carapace against sand was -0.33 ± 0.03 (n = 12). All contrast values were determined by reflection densitometry, using a reflection density guide (Kodak 146 5947) and panchromatic black and white film (Kodak Plus-X). The three different castings, together with three shades of paint, yielded nine experimental objects. Two of each object, giving a total of 18 castings, were placed in random order in a row 4 m below the high-water line on the south side of Mashnee Dike, Cape Cod. Massachusetts. We observed the behaviour of males in the vicinity of each casting during eight high tides, four at night and four during the day.

Figure 1a shows a male Limulus mounted on a black cement casting of the female carapace. The male has mounted the dorsal part of the casting in the usual position for egg fertilization. Some males maintained this position for several hours. Figure 1b shows a single male soon after it approached and made contact with the prosomal region of a grey casting. Figure 1c shows a male mounted on a white casting; four other males hover nearby, apparently searching for an opportunity to mount. The behaviour in Fig. 1 is indistinguishable from that of normal mating. Similar behaviour was observed in the vicinity of every cement casting, but the number of males around a specific casting depended on its form and contrast.

Figure 2a shows that the number of males was greatest around the female models and lowest around the cubes. In each of eight observation periods, males selected female models in preference to hemispheres and hemispheres in preference to cubes. The probability of these results occurring by chance is 3^{-8} ; p < 0.001. The degree of selection was not significantly influenced by time of day. We conclude that male Limuli can







Fig. 1 Male horseshoe crabs in the vicinity of submerged, painted castings of the female carapace. a, A male Limulus mounted on the posterior portion of a black casting in the normal position for egg fertilization. b, A male in contact with a grey casting soon after approaching the model. c, One male mounted on a white casting with four others hovering nearby apparently searching for the appropriate mating position. All photographs were taken during the same night-time observation period with Kodak Plus-X film and flash. The animals and models are under water in each photograph. Note in b the ripples generated on the water surface by the male. The overall length of the black casting in a is 55 cm.

discriminate form. Discrimination of form may be based on tactile or visual inputs, or both.

Figure 2b shows that the contrast of a casting also influences male mating behaviour. The data on male contacts with castings were pooled according to the shade of paint used (black, grey or white). Black castings yielded the highest percentage (40%) of male contacts, regardless of time of day. The attraction of males to the grey castings was also high and almost equal to that of black castings during the day but not at night. Black and grey castings may have attracted most males during the day because they are similar in shade to the female carapace and are clearly visible. Note that the relative contrast of the female carapace against sand is -0.33, which falls within the contrast values of the black and grey castings (-0.7 and +0.1). At night, the low relative contrast of the grey castings against sand may have made them less visible. Indeed, the grey Limulus casting in Fig. 1c is difficult to distinguish from its underwater background. We could not see the grey castings at night but had no difficulty in detecting the white and black castings by illumination from either a new or full moon. The results in Fig. 2b suggest that, when visibility is not a limiting factor, male Limuli are attracted to objects that appear similar in shade to the female carapace.

Taken together, the data in Fig. 2a and b show that males were attracted most to the black Limulus casting and least to the white cube: in each of the eight observation periods, black Limulus castings yielded the highest number of male contacts and white cubes the lowest number.

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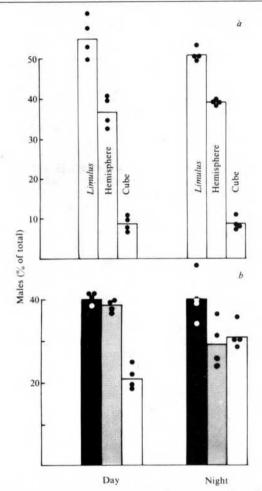


Fig. 2 Percentage of males observed at the cement castings during the day and at night. a. Filled circles indicate the percentage of males observed at each cement form during four daytime and four night-time observation periods. The daytime percentages did not differ significantly from the night-time values. Columns represent the average values. b, Filled and unfilled circles indicate the percentage of males in contact with the black (solid columns), grey (stippled columns) and white (open columns) castings during the same eight observation periods as in a. Columns represent the average values. The black castings attracted the greatest percentage of male Limuli regardless of time of day. The attraction of the grey castings was greater during the day, that of the white castings lower. Results are based on a total count of 6,988 males in contact with the set of 18 castings described in the text. The data were collected during eight high tides as males, in their search for females along the water's edge, approached and explored the submerged castings. Two observers separately surveyed the castings every 5 min during the 3-hr period that the tide covered the set of castings. The day- and night-time data were then summed and normalized.

Blinded animals were not attracted to the cement castings. In one night-time experiment we covered the median and lateral eyes of 75 male Limuli with black acrylic paint. The density of males around the castings fell by a factor of 8, and the distribution of the few animals found near the castings did not match that shown in Fig. 2. This result cannot be considered conclusive because some of the blinded animals buried themselves in the sand after being released at the water's edge. Nevertheless, the results are consistent with the notion that vision has a role in mating behaviour.

Visually guided behaviour is also evident from films taken during the day, which show that male Limuli passing within 2 m of a casting of a female carapace often change orientation and travel directly towards the casting, then explore and attempt to

Vision thus appears to play a part in Limulus mating behaviour, but other sense modalities may also be involved. Our

observations suggest that male animals are attracted to objects by form and contrast. Maintained contact with objects may be based on tactile cues transmitted by a well developed mechanoreceptor system7,8. Chemical cues may also be involved9, but as yet no information on chemical attractants is available.

Circadian rhythms in the Limulus visual system10 may be an integral part of the mechanisms underlying the behaviour reported here. At night a circadian clock in the Limulus brain transmits efferent neural activity to the lateral eyes, increasing the response and decreasing the noise of single photoreceptor cells11. Circadian changes in retinal physiology and anatomy increase the sensitivity of a dark-adapted lateral eye by as much as 30-100 times at night12. High visual sensitivity may adapt the animal for specific nocturnal behaviour such as mating.

We thank Mary Arabian for assistance. This research was supported by NSF grants BNS 8025519 and 8104669, and NIH grant EY-00667.

Received 8 October 1981; accepted 11 January 1982.

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Serotonin-activated adenylate cyclase during early development of Schistosoma mansoni

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Schistosoma mansoni infection is a major parasitic disease which afflicts 200 million people, mostly in developing countries. The parasite has a complex life cycle during which it is parasitic on a snail at the larval stage and on a mammalian host as an adult. In addition, there are two free-swimming stages: miracidia, which must penetrate the snail host, and cercariae, which leave the snail to find the correct mammalian host. On penetration into the mammalian host, the cercaria loses its tail and is referred to as a schistosomule. Adult stages of Fasciola hepatica (liver fluke)1,2 and S. mansoni3 have probably the highest adenylate cyclase activity reported, and in both parasites the enzyme can be activated by serotonin. Studies on Fasciola' have shown that GTP is necessary for serotonin activation. One possible main function of high adenylate cyclase activity is the regulation by cyclic AMP of anaerobic glycolysis, which is the main source of energy in the adult stage of these parasites5 We have compared the activities of adenylate cyclase in different developmental stages of Schistosoma. We report here that the cercariae have low adenylate cyclase activity which responds only poorly to activation by serotonin and GTP. However, in schistosomules cultures in vitro7, we found that within the first 4 days the cyclase system changed from one showing low serotonin activation, similar to that of cercariae, to one of high activity similar to that found in the adults.

Adenylate cyclase activity in adults was compared with that in cercariae. Particle preparations isolated at 2,000g contained

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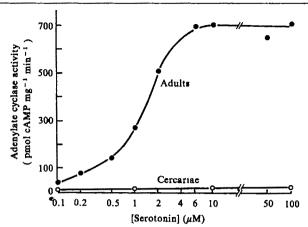


Fig. 1 Serotonin activation of adenylate cyclase from adult and cercaria particles. Representative experiment on adult (●) and cercaria particles (○) in which serotonin was added at the indicated concentrations to adenylate cyclase reaction mixture containing 0.1 mM ATP and 1×10⁻⁵ M GTP. Adenylate cyclase activity was determined as described in Table 1. Basal activity (without activators) for adults was 8 pmol cyclic AMP (cAMP) mg⁻¹ min⁻¹ and <3 pmol cyclic AMP mg⁻¹ min⁻¹ for cercariae.

almost all the cyclase activity. Representative experiments summarized in Table 1 show that when the enzyme was maximally activated with NaF, the specific activity of the cercarial enzyme was $\sim 20\%$ of that of the adult. Serotonin plus GTP caused almost no activation of the cercarial enzyme, whereas it markedly stimulated adult adenylate cyclase. Figure 1 shows the effect of various concentrations of serotonin plus GTP on the enzyme from cercariae and adults. The specific activity of the two enzymes is markedly different even at concentrations as low as $1-10~\mu\mathrm{M}$ of serotonin. The results in Table 1 also show that the poorly hydrolysed GTP analogue, Gpp(NH)p (guanylyl imidophosphate), by itself stimulated the enzyme from both sources, whereas the natural nucleotide GTP had no stimulatory effect. Note that serotonin in the presence of Gpp(NH)p caused significant stimulation of enzyme activity

Table 1 Cyclic AMP (pmol mg⁻¹ min⁻¹) produced in cercariae and adult S. mansoni

Activators	Cercariae	Adults
None	2	8
NaF (10 mM)	1,232	5,985
GTP (0.01 mM)	4	. 7
Serotonin (100 µM)		
Serotonin (100 μ M) + GTP (×10 ⁻⁵ M)	15	557
$Gpp(NH)p (2 \times 10^{-5} M)$	66	440
Gpp(NH)p $(2 \times 10^{-5} \text{ M})$ Gpp(NH)p $(2 \times 10^{-5} \text{ M})$		
+Serotonin (100 µM)	213	1,798

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Adult worms, obtained from hamsters by perfusion 16, were homogenized using a glass homogenizer in 0.25 M sucrose, 5 mM dithiothreitol (DTT) and 1 mM EDTA. Snalls were placed under light to induce shedding of cercariae. Cercariae were concentrated by cooling until swimming stopped, and then centrifuged. The pellet was homogenized in a glass homogenizer with a motorized glass-Teflon pestle in the same buffer as the adults. Both homogenates were spun twice at 2,000g for 15 min each. The final resuspended particles were used as the enzyme source. We determined adenylate cyclase activity using the method of Salomon *et al.*¹⁷, which measures the production of radio-actively labelled cyclic AMP from $\left[\alpha^{-32}P\right]ATP$. ³H-labelled cyclic AMP was added to the mixture after the reaction was stopped to monitor cyclic AMP recovery as described by Salomon et al. 17 under 'method . Assays contained 0.1 M sucrose, 50 mM glycylglycine (pH 7.5), 0.5 mM isobutyl methylxanthine, 5 mH phosphocreatine, 2 mM MgCl₂, 5 mM DTT, 0.02 mM EDTA, 0.1 mM Na₂ATP, 5 U creatinephosphokinase, and \sim 1 μ Ci [α -³²P]ATP. Reactions were initiated with 0.05 ml of particles and the final volume of the reaction mixture, with or without specific activators, was 0.25 ml. All samples were incubated for 10 min at 30 °C. Protein was determined by the method of Bradford 18. from the cercariae, though not as high as that of the adult. In these conditions of enzyme activation, the specific activity of the cercarial enzyme never exceeded 15% of that of the adult.

As there was such an obvious difference in total adenylate cyclase activity (with NaF) and serotonin-stimulated activity between adults and cercariae, the question arose of how rapidly the enzyme develops from low to high responsiveness to serotonin. We therefore studied the enzyme activity in the early intermediate stages of development from cercariae to adults. Schistosomules were prepared and cultured at 36 °C in a 5% CO₂ atmosphere for 10 days according to the procedure of Basch⁷, in medium containing 10% serum and 1 µM serotonin. Representative results for cyclase activity at different stages of development of the schistosomules are shown in Fig. 2. NaF-stimulated adenylate cyclase activity increased after preparation of the schistosomules from the cercariae; such an increase does not appear to be due to the experimental procedure used as preparation of the schistosomules by mechanical means⁸ without exposure to tissue culture media gave the same increase in fluoride-activated enzyme. The most significant change in the adenylate cyclase activity during schistosomule development was the marked increase in serotoninactivated enzyme on the second and fourth days (Fig. 2). While serotonin-activated adenylate cyclase in the cercariae was only 0.8% of NaF-stimulated activity, by the fourth day the adenylate cyclase in the schistosomules could be activated by serotonin to 6% of NaF-stimulated activity. Adenylate cyclase activity of schistosomules that were maintained in culture for up to 10 days showed no further increase in sensitivity to serotonin activation. Enzyme from adult worms grown in vitro also showed activation by serotonin to 6% of total activity (NaF-stimulated), although total activity doubled (Fig. 2). Serotonin-activated cyclase activity was higher in the parasites grown and collected from the hamster host than in schistosomules or in adults grown entirely in vitro (Fig. 2).

We then tested the effect of serotonin activation in the presence of Gpp(NH)p. Figure 3 shows that serotonin in the presence of Gpp(NH)p caused significant activation of cyclase in cercariae and schistosomules, an effect not shared by the natural nucleotide, GTP. Further activation by serotonin had doubled by the second day of schistosomule culture, when it reached a plateau. Note that much of the serotonin activity in

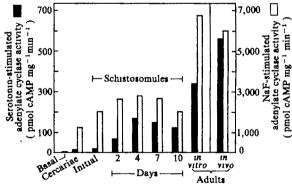


Fig. 2 Activation of adenylate cyclase by serotonin and GTP (left ordinate) and by NaF (right ordinate) at different stages of development. Cercariae from infected snails and adults from hamsters were collected as described in Table 1. Early schistosomules (initial) were isolated from cercariae and cultured according to the procedure of Basch⁷. Adults were prepared in vitro after culturing the schistosomules for 3 months, according to the Basch procedure. Preparation of particles as well as assay of adenylate cyclase were as described in Table 1 legend. Adenylate cyclase activity was measured in a reaction mixture containing 0.1 mM ATP and either 10 mM NaF or 100 µM serotonin with 10⁻⁵ GTP. Adenylate cyclase activity in particles from schistosomules immediately after preparation from the cercariae is indicated by 'initial'. Basal activity for adults was <8 pmol cyclic AMP mg and for cercariae and schistosomules <3 pmol cyclic AMP $mg^{-1} min^{-1}$.

the presence of Gpp(NH)p in these stages of early development is due to the effect of the nucleotide itself. The adenylate cyclase activity of the adult worm (grown either in vitro or in vivo) was still higher than that in the early stages of development, indicating further increase in enzyme activity during development of the parasite to maturity. Serotonin has been reported to stimulate the motility of several trematodes including schistosomes (for review see ref. 9). In the present experiments we observed that cercariae, immediately after shedding their tails, did not respond to the stimulatory action of serotonin. However, after only 2 h in tissue culture medium, schistosomules responded fully to the stimulatory action of the indolamine on motility.

The cercariae of this trematode are short-lived free-living larvae whose main function is to find a suitable warm-blooded host. The results reported here clearly show that at this stage adenylate cyclase activity and its responsiveness to serotonin are extremely low. While the cercaria is a free-living organism in water, it develops to a schistosomule in the host, where it encounters serotonin. We have shown that early in schistosomule development, adenylate cyclase activity becomes higher and its responsiveness to serotonin markedly increases. The adult parasite showed an even greater increase in both total- and serotonin-stimulated adenylate cyclase activity. Thus as the parasite develops to maturity, it gains more adenylate cyclase that is sensitive to serotonin activation. Because of the marked difference between fully serotonin-activated cyclase activity and the maximum capability of the enzyme system in both schistosomules and adults, it is possible that other naturally occurring factors besides serotonin control cyclase activity during development of the parasite. The results suggest that adenylate cyclase is necessary to supply cyclic AMP early in development so as to achieve full parasite maturation. Cyclic AMP has been reported to be important for the development of trypanosomes10 and malarial parasites11, as well as for cell differentiation¹² and proliferation¹³.

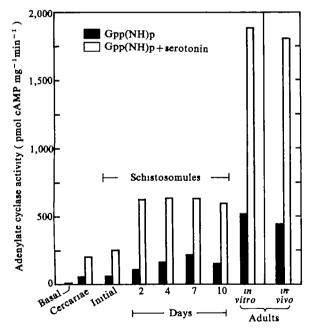


Fig. 3 Activation of adenylate cyclase from schistosomules by Gpp(NH)p with and without serotonin. Representative experiment on cercariae, schistosomules and in vitro and in vivo adults. Schistosomules and adult worms (in vitro) were obtained from cultures, cercariae were obtained from infected snails as described in Table 1, and adults (in vivo) were dissected from infected hamsters. Particles were prepared and analysed as described in Table 1 legend. All adenylate cyclase reaction mixtures contained 0.1 mM ATP and 2×10^{-5} M Gpp(NH)p with or without 100 μ M serotonin, as indicated in Table 1. Basal activity for adults was <8 pmol cyclic AMP mg⁻¹ min⁻¹ and for cercariae and schistosomules <3 pmol cyclic AMP mg⁻¹ min⁻¹.

We reported previously that serotonin activates three key enzymes in the regulation of metabolism in a closely related parasite, F. hepatica: glycogen phosphorylase¹, phosphofructokinase⁶ and cyclic AMP-dependent protein kinase¹ all these enzyme systems, cyclic AMP appears to be involved in enzyme activation and therefore may be crucial for normal development of the parasite in the host. Apparently, the schistosomule synthesizes an adenylate cyclase that is more responsive to serotonin than the enzyme in cercariae. This developmental stage occurs in the first 48-96 h of the schistosomule life cycle, which corresponds closely to the time of complete skin penetration¹⁵ and arrival in the vascular system where serotonin is present. Serotonin-activated adenylate cyclase in the early stages of schistosome development offers sites which are amenable to pharmacological manipulation and which could be used for the selection of more effective antischistosomal agents.

This work was supported by USPHS research grants MH 23464 and AI 16501. We thank Dr Paul Basch and Mrs Natalicia Basch for providing us with infected snails, infected hamsters and schistosomules.

Received 12 October 1981; accepted 26 January 1982.

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Expression in E. coli of Chlamydia trachomatis antigen recognized during human infection

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Chlamydia trachomatis, a prokaryotic obligate intracellular parasite of eukaryotic cells, has long been recognized as the agent of trachoma, the major infectious cause of human blindness1. In the past decade, clinical investigations have shown that the frequency and severity of chlamydial genital infection parallels that of gonorrhoea, and that C. trachomatis is a major cause of non-gonococcal urethritis, epidymitis, proctitis, cervicitis and salpingitis, as well as conjunctivitis and pneumonia in neonates². Despite the new awareness of its role in human disease, there is a need for improved methods of identification and control of C. trachomatis infections. Progress in these areas awaits a more detailed understanding of the pathogenesis of chlamydial infection, including definition of key antigenic structures recognized in human disease. We have studied the immune response to chlamydial polypeptides during human infection and have found that most infected patients have antibody to polypeptides of molecular weight (M_r) 67,000 (67K), 60K, 40K, 19K and 16K, regardless of infecting immunotype3. We report here the cloning and expression in Escherichia coli of the 19K C. trachomatis polypeptide, an immunogen in human chlamydial infection.

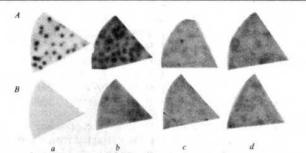


Fig. 1 Clone A4 plaques react specifically with serum from humans having chlamydial infection. Plates containing 100–150 clone A4 plaques were overlaid with nitrocellulose disks for 10–20 min. The disks were cut into quadrants then soaked in 5% OA–TSA (5% ovalbumin in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.15% sodium azide) to saturate unused protein binding sites, and incubated overnight with individual sera diluted 1:250 in 1% OA–TSA. After washing, the disks were reacted with ¹²⁵I-protein A, washed, dried and exposed to Kodak X-Omat AR film between intensifying screens. A, reaction of A4 plaques with sera from infected (a, b) and uninfected individuals (c, d). B, reaction of A1059 plaques with the same four sera.

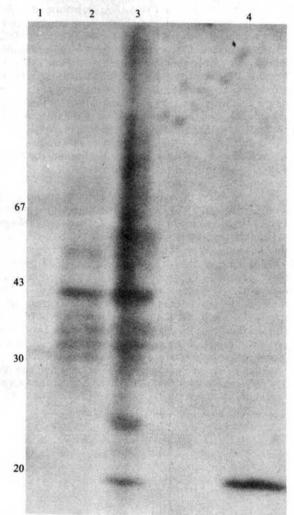


Fig. 2 Autoradiogram of clone A4 'Western' blot. Lysate samples were boiled in 0.0625 M Tris, 2% SDS, 10% glycerol, 0.001% bromophenol blue, 5% β-mercaptoethanol before loading on to 8-20% gradient polyacrylamide SDS gels. After electrophoresis and transfer to nitrocellulose, the blot was probed with the human chlamydial serum used in earlier screening (see Fig. 1a). Radioiodinated molecular weight standards (Pharmacia) are marked on the left ($×10^3$). Samples shown are host $E.\ coli\ Q359$ (lane 1), chlamydial L_1 elementary bodies (lanes 2, 3), and clone A4 grown in broth cultures of Q359 (lane 4). Except for lane 2, which was an older, partially degraded sample, approximately equal amounts of protein were applied to each lane.

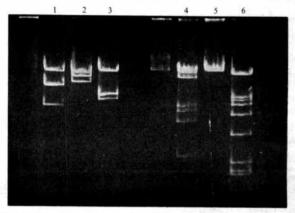


Fig. 3 Restriction endonuclease digests of vector and clone A4 DNA. DNA extracted from purified vector or recombinant phage was digested with various restriction endonucleases in recommended buffers (New England Biolabs). Cleaved DNA samples were then analysed on a 0.6% agarose gel and stained with ethidium bromide. Shown here are λ1059 digested with EcoRI (lane 1), BamHI-digested λ1059 (lane 2), λ1059/HindIII (lane 3), A4/EcoRI (lane 4), A4/BamHI (lane 5), A4/HindIII (lane 6). Fragments were sized using λ/HindIII markers (BRL; data not shown). Unlabelled lanes contain nonreactive clone plaques.

Elementary bodies (EB) of *C. trachomatis* type L₁, grown in HeLa cells, were purified through Renografin (Squibb) to yield EB free from host cell contamination⁴. Chlamydial DNA was extracted from EB in 20% sucrose containing 50 mM Tris pH 7.5, 20 mM Na₂EDTA, 200 μ g ml⁻¹ proteinase K, and 0.7% Sarkosyl at 55 °C for 15 min, followed by further incubation at 37 °C for 45 min. After phenol extraction and equilibrium centrifugation in neutral CsCl, the chlamydial DNA was dialysed against 10 mM Tris pH 7.5, 0.1 mM Na₂EDTA. It was then partially digested with Sau3A I and ligated to BamHI-cleaved vector phage λ 1059 (ref. 5). The ligated DNA was then packaged in vitro⁶ and used to transfect E. coli Q359 which is permissive only for recombinant plaques. Plaques were screened for expression of chlamydial antigens by an in situ radioimmunoassay.

Nitrocellulose disks were placed over the recombinant bacteriophage plaques to absorb plaque-derived protein. The disks were then exposed to serum from a human with chlamydial infection, followed by reaction with ¹²⁵I-labelled protein A of Staphylococcus aureus (Pharmacia) and autoradiography. One plaque which gave a particularly strong signal, designated A4, was chosen for further study (Fig. 1). Plaques of clone A4 reacted specifically with sera from patients having chlamydial infection but showed no reaction with sera from uninfected humans in conditions where plaques of vector λ 1059 (without a chlamydial DNA insert) did not react with sera from patients with chlamydial infection or from control patients.

The nature of the chlamydial antigen encoded by clone A4 was elucidated by 'Western blotting' of total protein from an A4 lysate (Fig. 2). A4 specifies a polypeptide antigen of M_r 19K (Fig. 2, lane 4), identical in size to that of an antigen of C. trachomatis elementary bodies.

DNA from clone A4 was partially characterized by restriction endonuclease cleavage (Fig. 3). As expected, clone A4 has a cleavage pattern which differs from that of the original vector $\lambda 1059$, consistent with an insert size of 15 kilobase (kb).

We have described here the expression in E. coli of a M_r 19 K antigen of C. trachomatis; we have shown previously³ that an antigen of this molecular weight is a consistent immunogen in human chlamydial infection. Antibody to this polypeptide is therefore indicative of chlamydial infection regardless of immunotype, and this could possibly become the basis of simple serotests for chlamydial infection. We believe that cloning the genes for major chlamydial immunogens in human infection provides a powerful tool for studying the immunobiology of this organism. It should be possible, using recombinant sources of

chlamydial antigens, to reproduce by experimental vaccination the pattern of antibody to chlamydial polypeptides found in various chlamydial diseases, and then determine whether this modifies experimental chlamydial disease.

This work was supported by a gift from the Cetus Corporation to M.A.L. W.M.W. received a Canadian MRC fellowship until July 1981 and is now a Scholar of the Alberta Heritage Foundation for Medical Research.

Received 23 November 1981, accepted 4 January 1982

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Transformation of the green alga Chlamydomonas reinhardii with yeast DNA

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The development of a transformation system in the green unicellular alga Chlamydomonas reinhardii is important for two reasons. First, a large number of nuclear mutants of this organism have been characterized, including auxotrophs, cytoplasmic and chloroplast ribosome mutants, photosynthetic and flagellar mutants^{1,2}. The isolation of wild-type genes from C. reinhardii and from other plants may be possible by complementation with an efficient transformation system. Second. modification and reinsertion of these genes and their regulatory elements into C. reinhardii cells is likely to provide important insights into their function and regulation. Here, we describe the first successful nuclear transformation of C. reinhardii. A plasmid carrying the yeast arg4 locus and a yeast replication origin^{3,4} has been used to transform a C. reinhardii cell walldeficient, arginine-requiring mutant. In several transformants the yeast gene appears to have integrated stably into the nuclear

The arg7 locus of C. reinhardii was used for selection. It codes for argininosuccinate lyase (ASL), the last enzyme of the arginine biosynthetic pathway, which converts argininosuccinate into arginine and fumarate⁵. In C. reinhardii the enzyme consists of five (±1) identical subunits of molecular weight ~39,000 (ref. 6). The first arg7 mutant of C. reinhardii was isolated by Gillham⁷; several other mutants of this type have since been obtained and characterized8.9. The DNA used for transformation was the plasmid pYearg4 containing a yeast replication origin and the yeast arg4 locus^{3,4}, which is analogous to the C. reinhardii arg7 locus. The 18 kilobase (kb) pYearg4 plasmid contains a single EcoRI site and eight HindIII sites and the arg4 gene is located on the second largest (2.7 kb) HindIII fragment³ (fragment B in Fig. 2h). One important advantage of using this plasmid is that it cross-hybridizes only weakly with nuclear DNA of C. reinhardii, thereby allowing an easy distinction between true transformants and revertants (see below). To avoid using cell wall-degrading enzymes to obtain protoplasts, the cell wall-deficient strain cw15 (ref. 10) was chosen. The double mutant cw15 arg7 was constructed by Dr P. Bennoun.

Cells of cw15 arg7 were treated with poly-L-ornithine¹¹ or polyethylene glycol (PEG), mixed with yeast plasmid and plated on plates containing acetate as sole carbon source¹² (see Fig. 1 legend for experimental details). After 2 weeks small green colonies appeared at a frequency of $\sim 10^{-6}$. Table 1 shows that the poly-L-ornithine method is more reproducible than the PEG method.

As the reversion frequency of arg7 is $\sim 10^{-7}$ (ref. 9), the putative transformants were tested for the presence of yeast plasmid sequences. Total DNA was extracted from these cells and digested with restriction endonucleases. The DNA fragments were electrophoresed on agarose gels (Fig. 1), transferred to nitrocellulose filters according to Southern¹³ and hybridized with radioactively labelled pYearg4 plasmid DNA. Figure 1b shows that a 13.1 kb EcoRI fragment hybridizes with the probe in transformant T2-5. Hybridization of the same probe with undigested DNA produces a smear (Fig. 1d), indicating that the plasmid DNA is integrated in the nuclear DNA of this transformant. The possibility that the plasmid is not integrated but present as a high molecular weight concatemer can be excluded because pYearg4 hybridizes to a nuclear HindIII fragment that is larger than the largest HindIII fragment of pYears4 (Fig. 2) lanes f, h). When total DNA from non-transformed cells is hybridized with the same probe, only one faint band, of lower molecular weight, is apparent (Fig. 1f). When nuclear DNA from non-transformed cells was mixed with one copy equivalent of pYearg4 plasmid per genome, cleaved with EcoRI (Fig. 1g) and hybridized to the probe (Fig. 1h) in order to estimate the copy number of integrated sequences, the intensity of the signal was comparable with that of Fig. 1b. Comparison of the sizes of pYearg4 (Fig. 1h) and the EcoRI fragment of the transformant, which hybridizes to the probe (Fig. 1b), reveals that at least 5 kb of the plasmid DNA have been deleted in the transformant. Similar hybridization patterns are obtained when the ColE1 vector alone or the purified HindIII fragment B of pYearg4 are used as probes (data not shown).

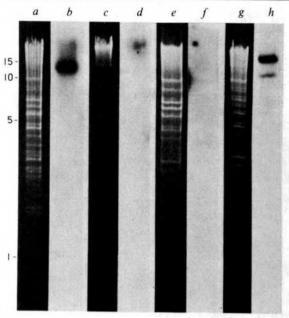
Figure 2a-c displays the hybridization patterns obtained with another transformant, T8-3. Whereas one major signal is visible with EcoRI-(Fig. 2a) or HindIII- (Fig. 2c) digested DNA, no strong band is detectable with undigested DNA (Fig. 2b), probably because of the poor transfer of larger fragments of DNA. As in the previous case it can be concluded from these data that a portion of the yeast plasmid has inserted itself into the nuclear DNA. Note that the EcoRI fragment of T8-3 hybridizing to the probe differs in size from the corresponding fragment of T2-5 and that the hybridization patterns obtained with HindIII digests of the DNAs of the two transformants are different (Fig. 2c, e, g). It appears therefore that the integration of pYearg4 in the two transformants has either taken place at different sites in the nuclear genome or at the same site with different deletions and/or that rearrangements of the plasmid sequences have occurred.

Table 1 Summary of transformation experiments

Expt	DNA used for transformation (5 µg)	No of a Method I	arg ⁺ cells Method II
1	pYearg4	3	
2	pYearg4	2	3
3	pYearg4	2	0
4	pYearg4	1	0
5	pYearg4	4	0
7	pYearg4	4	0
8	pYearg4	3	2
9	pBR322-arg4	1	0

Methods I and II refer to the poly-L-ornsthine and PEG transformation methods (see Fig. 1 legend). For each transformation 2×10^7 cells of cw15 arg7B were used. Cell survival was 50-80% in method I and 0-40% in method II. The pBR322-arg4 plasmid is described in Fig. 2 legend. In all cases 50 μg of salmon sperm DNA were included. In control experiments in which plasmid DNA was omitted, no Arg+ cells were recovered. Transformants T2-5 and T8-3 used in this study were obtained in experiments 2 (method II) and 8 (method I) respectively. Approximately one-half of the Arg+ cells examined by the Southern blotting technique displayed fragments hybridizing to the yeast DNA.

Fig. 1 Analysis of transformant T2-5. Two methods of transformation were used. I: 2×10^7 cells of arg7 cw15B at a concentration of $< 2 \times 10^6$ cells per ml were centrifuged at 3,000g for 5 min, washed once in Tris-acetate-phosphate (TAP), 0.2 M mannitol¹² and resuspended in 0.95 ml TAP, 0.4 M mannitol. Five to ten µg of pYearg4 plasmid DNA3 were preincubated with 50 μg carrier salmon sperm DNA and poly-L-ornithine in 5 mM ZnSO₄15 for 1 h on ice before being added to the cells. The final poly-L-ornithine concentration was 10 µg ml-1 and incubation was for 30 min at room temperature. Cells were washed three times in 5 ml TAP, 0.4 M mannitol; resuspended in 10 ml TAP, 0.2 M mannitol, 50 µg ml⁻¹ arginine and placed on a shaker at 150 r.p.m. for 20 h under a light intensity of 3,000 lx. The cells were concentrated to 1 ml and 0.2 ml aliquots were mixed with 3 ml top agar (0.6% agar in TAP, 0.2 M mannitol)16 at 37 °C and poured on TAP plates (1.5% agar). Controls were treated identically except that the plasmid DNA was omitted. II: The second method is similar to the yeast transformation procedure¹⁷. After resuspension of the cells in 0.2 ml 10 mM CaCl₂ in TAP, 0.4 M mannitol, plasmid DNA (5-10 μg) and carrier DNA (20-50 μg) were added and the mixture was incubated at room temperature for 15 min. Two ml of 30% PEG-4000 in 10 mM Tris-HCl pH7.5, 10 mM CaCl₂ were added and the cells were incubated for 30 min at room temperature. The cells were washed and plated as in method I. The nuclear DNA was isolated from a 500 ml stationary phase culture. Cells were washed once with TAP growth medium¹² and resuspended in 0.1 M NaCl, 20 mM Tris-HCl pH8.0, 50 mM EDTA. SDS and pronase were added at a final concentration of 1% and 500 µg ml-1 respectively. Fresh pronase was added after 45 and 90 min. After 2.5 h incubation at 50 °C the lysate was cooled and extracted with distilled phenol. The aqueous phase was precipitated with 2 vol ethanol and centrifuged for 10 min at 10,000g. The pellet was washed once with 70%



ethanol, dried and resuspended in 2 ml 10 mM Tris-HCl pH8.0, 1 mM

EDTA overnight. The preparation was then treated with 50 μg ml⁻¹ pancreatic RNAse for 1 h at 37 °C. The DNA was centrifuged in an ethidium bromide density gradient (Beckman Ti50 rotor, 50 h at 35,000 r.p.m.) after inactivation of the enzyme with 0.05% diethyl pyrocarbonate for 5 min at room temperature. The DNA band was removed with a pipette, extracted twice with isoamylalcohol, precipitated with 3 vol 70% ethanol, rinsed in 70% ethanol and resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. The agarose gel electrophoretic patterns are a, DNA of transformant T2-5 digested with EcoRI; c, T2-5 DNA without digestion; e, DNA from non-transformed cells digested with EcoRI; g, DNA from non-transformed cells mixed with pYearg4 DNA digested with EcoRI. The DNA fragments were blotted onto nitrocellulose filters¹³. b, d, f, h Represent autoradiographs of hybridizations of the DNAs shown in a, c, e, g respectively, using nick-translated¹⁸ pYearg4 plasmid DNA as a probe. The discrete bands in a, e and g represent chloroplast EcoRI fragments and the band in c, ~15 kb, is mitochondrial DNA^{19,20}, Numbers on the left indicate the sizes of the fragments in kb. Hybridizations were performed as described previously²¹ with minor modifications. Nitrocellulose filters carrying DNA fragments (16 × 16 cm) were prehybridized in 6 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate pH 7.0), 1 mM EDTA, 0.1% SDS, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 μg ml⁻¹ salmon sperm DNA at 68 °C for 2-4 h. Hybridizations were in the same medium at 68 °C for 16-20 h except that the salmon sperm DNA concentration was raised to 1 mg ml⁻¹; 3 × 10⁶ c.p.m. of ³²P-labelled hybridization probe were added. Filters were washed twice in 2 × SSC, 0.1% SDS at room temperature; four times for 1 h in 0.1 × SSC, 0.1% SDS at 52 °C; and 10 min in 3 mM Tris-base at room temperature. Filters were dried and exposed to Kodak Xs-5 films with tungsten intensifyin

Comparison of the sizes of the *HindIII* fragments of pYearg4 (Fig. 2h) with the hybridization patterns obtained with the *HindIII* digests of the DNAs from the transformants T8-3 (Fig. 2c) and T2-5 (Fig. 2f, g) confirms that a deletion exists in the integrated plasmid DNA relative to the free plasmid. As no hybridization occurs at the position of fragment B which contains the arg4 locus, we conclude that a portion of this fragment has been fused to a nuclear DNA fragment. The fate of the smaller *HindIII* fragments of pYearg4 is more difficult to follow in the integrated state because the hybridization signals obtained with the probe are at the limit of detection.

Table 2 shows that the two transformants examined grow much more slowly than the non-arginine-requiring strain cw15. It also shows that the specific activity of ASL in crude extracts of the cw15 strain was 0.14 µmol arginine per mg protein per h, considerably lower than the value reported for wild-type cells9. The activity of this enzyme is known to increase when the cells are grown in medium containing low concentrations of ammonium9; however, our cw15 strain and the transformants grow very poorly in these conditions and it was therefore necessary to use high concentrations of ammonium (400 mg l-1) which repress the activity of the enzyme8. In addition, the enzyme may be subjected to more proteolysis due to the fragility of the cw 15 mutant cells. Table 2 shows that the ASL activities in the transformants are close to 5% of the value obtained in the cw15 strain, and are at the limit of detection. The yeast enzyme appears therefore to have a low activity in C. reinhardii and/or be unstable in these cells. It is known, however, that C. reinhardii can sustain a normal growth rate even if the level of ASL is low. While examining the interallelic complementation of several mutants of the arg7 locus of C. reinhardii, Loppes and Matagne9 found one diploid with a normal growth rate although the specific activity of ASL was only 5% of wild type. Besides interallelic complementation, it is also known that hybrid ASL can be formed between the products of wild-type and certain arg7 mutant genes14. The question arises whether the ASL enzyme of the transformants consists uniquely of yeast subunits

or whether it is made functional by the interaction of the yeast and mutated *C. reinhardii* subunits. Analysis of the heat sensitivity of the enzyme may distinguish between these two possibilities¹⁴, but we have not yet been able to perform such an experiment because of the low specific activity of the ASL enzyme in the transformants.

The stability of the transformants was examined by growing them for several generations in the presence of arginine. After 70 generations without selective pressure cells of T2-5 were still arginine independent. However, analysis of the integrated DNA by Southern blotting revealed changes in the hybridization pattern (Fig. 2). After 70 generations the EcoRI (compare Fig. 2d, e) and HindIII (compare Fig. 2f, g) fragments hybridizing to the probe have become smaller, suggesting that a segment of the integrated DNA has been deleted or that there has been a slight sequence rearrangement.

Because the transformation yield is still low it has been difficult to optimize the transformation procedure. It is not yet clear whether the limiting step is at the level of entry of DNA into the cells or at the level of stable integration of the DNA into the nuclear genome. Although the pYearg4 plasmid has been shown to contain a yeast replication origin and to generate high-frequency transformants in yeast⁴, we have no solid evidence that this replication origin is active in *C. reinhardii*. Note,

Table 2 Properties of transformants

Specific activity of ASL (µmol arginine per mg protein per h)	Generation time
_	14
0.14	7
0.01	17
0.01	23
	of ASL (µmol arginine per mg protein per h) 0.14 0.01

The specific activities of ASL in crude extracts were determined as described in ref. 8. Cells were grown in Tris-acetate-phosphate (TAP) medium except cw15 arg7 cells which were grown in TAP medium containing 50 μ g ml⁻¹ arginine.

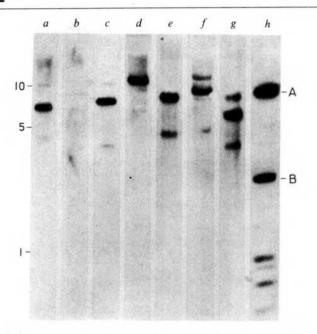


Fig. 2 Analysis of transformants. Preparation of the DNA of the transformants and the hybridizations of the DNA fragments with ³²P-labelled probes were performed as described in Fig. 1 legend. To obtain more specific hybridization to the arg4 region, the HindIII fragment containing arg4 (fragment B in lane h) was subcloned into pBR322. The new plasmid pBR322-arg4 was nick-translated and used as probe in the hybridizations shown in a-c, e, g. The hybridization probe used in d, f and h was pYearg4. Lanes a, b, c represent autoradiographs of hybridizations of the probe to DNA of the transformant T8-3: a, EcoRI digest; b, undigested DNA; c, HindIII digest. Lanes, d, f display hybridization patterns obtained with the DNA of T2-5 digested with EcoRI and HindIII respectively. Lanes e, g represent similar hybridizations with EcoRI and HindIII digests except that the DNA was extracted from T2-5 cells about 70 generations later than in d and f. Lane h displays the HindIII fragments of pYearg4. Fragments A and B contain the ColE1 vector and the yeast arg4 locus respectively3. Numbers on the left indicate the sizes of the fragments in kb

however, that two putative slow growing transformants were shown initially, by the Southern blotting technique (data not shown), to harbour non-integrated DNA molecules hybridizing with the pYearg4 plasmid. After further growth of these clones subsequent analysis failed to show any DNA fragment hybridizing to the probe. It is possible that the strains initially carried an autonomously replicating plasmid, which was lost after reversion to arginine independence.

Despite these uncertainties the experiments described here clearly show that transformation is feasible in C. reinhardii and that some of these transformants can be stably propagated. It will be important to develop suitable transformation vectors for C. reinhardii in order to allow transformation to become an efficient method for isolating nuclear genes by complementation of appropriate mutants and for studying the regulation of expression of nuclear genes of C. reinhardii and other plants.

We thank M. Chandler for helpful comments, O. Jenni for photography, and Dr J. Carbon for plasmid pYearg4. This work was supported by grant 3.659.0.80 from the Swiss National Foundation.

Received 19 October 1981; accepted 18 January 1982.

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In vitro transformation of plant protoplasts with Ti-plasmid DNA

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Genetic engineering requires a procedure for introducing DNA into host cells, followed by integration into the host genome and gene expression. Although several procedures for DNA-mediated gene transfer in mammalian cells, yeast and bacteria have been reported, no such methods are yet available for plant cells. The major obstacle to DNA uptake in plant cells is the cell wall, but this can be circumvented by using plant protoplasts, cells freed of their cell walls by enzymatic digestion. However, none of the reports on the uptake of DNA into plant protoplasts 1,2 has produced conclusive evidence for the integration of DNA into the host genome, that is, that stable transformation occurs. The tumour-inducing bacterium Agrobacterium tumefaciens, which causes crown gall disease, is a natural system for the introduction of foreign DNA into plants. This bacterium introduces part of its tumour-inducing (Ti) plasmid, called T-DNA, into plant cells, where it becomes integrated into the nuclear DNA of the host3 and is transcribed into mRNA5.6. T-DNA encodes tumourspecific enzymes responsible for the formation of amino acid derivatives such as octopine or nopaline7, which the bacterium can use as a sole source of carbon and nitrogen. The transformed cells have also acquired the ability to grow in the absence of phytohormones (autotrophy). An in vitro system for infection of Nicotiana tabacum protoplasts by A. tumefaciens has already been reported. Transformants are selected by their ability to divide and grow in tissue culture without the addition of plant phytohormones to the synthetic culture medium. Here, we report a reproducible method for the stable transformation of tobacco protoplasts with Ti-plasmid DNA, using a similar selection procedure.

Protoplasts, isolated from leaves of aseptically grown N. tabacum SR₁ shoots, were cultured in K₃ medium supplemented

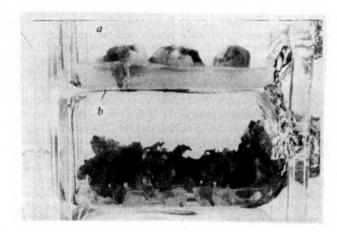


Fig. 1 Two callus lines obtained after in vitro DNA transformation growing in tissue culture on hormone-free LS medium. a, A typical non-regenerating line containing LpDH activity; pT2-15. b, A typical line that regenerates shoots, but lacks LpDH activity; pT2-6.

with phytohormones⁸ (see Table 1 legend). Normally, they form a new cell wall, divide and generate cell colonies (calli) which will eventually give rise to shoot-regenerating calli. In the present study transformants were selected on the basis of their phytohormone-independent growth. Other markers for the identification of transformants are the presence of lysopine dehydrogenase (LpDH) activity and T-DNA. Lysopine de-



Fig. 2 A mature, flowering plant obtained after grafting of a shoot derived from tissue line pT₂-2 on a stem of N. tabacum var. Samson⁹. Note the extended stamens of the flowers.

Table 1 Properties of tissues obtained after incubation of SR₁ protoplasts with pTi-Ach5 DNA

Tissue	Autotrophy	LpDH activity	Shoot regeneration
pT ₂ -2, 6, 8, 20*, 2	1* +	-	+
pT ₂ -9, 10, 11, 13, 1		+	_
pT ₂ -3	_	+	
$pT_7-2, 3$	+	+	-

N. tabacum SR₁ protoplasts (pps) were isolated from sterile shoots and suspended in K_3 medium containing 0.4 M sucrose, supplemented with naphthaleneacetic acid (0.1 mg I^{-1}) and kinetin (0.2 mg I^{-1}) at a density of $5 \times$ 105 pps per ml (ref. 8). (All conditions used in the isolation of pps and the culture of SR, shoots are described in ref. 8.) Fractions (1 ml, that is, 5×10^5 pps) were taken and 0.5 ml of polyethylene glycol (PEG 6000, 40% w/v) dissolved in a fusion medium11 (F) containing 140 mM NaCl, 5 mM KCl, 0.75 mM Na2HPO4, 5 mM glucose and 125 mM CaCl₂·2H₂O pH 7.0 was added, followed by 10 µg of pTiAch5 DNA (0.4 mg ml⁻¹) and 50 µg of CT-DNA (1 mg ml⁻¹). Massive clumping of the protoplasts occurred. Protoplasts were incubated at 26 °C for 30 min with occasional shaking, after which 10 ml F-medium was added stepwise in 2-ml portions at 5-min intervals. Aggregates broke up during this post-incubation. The protoplasts were pelleted by centrifugation and the supernatant was removed. The pellet was resuspended in 10 ml of K_3 medium and plated in 10-cm Petri dishes after addition of carbenicillin ($250 \mu g \text{ ml}^{-1}$). They were kept in the dark for 24 hand from then on in 2,000 lx for 12 h per day. Cell survival was $\ge 50\%$. Two weeks later, 5 ml of fresh K_3 medium was added. If the cell colonies were of sufficient size, they were plated in K3 medium containing 0.3 M sucrose solidified with 0.3% agar and still supplemented with phytohormones. After ~1 month on this medium the small calli were placed for selection on hormone-free K3 medium containing 0.2 M sucrose and 0.5% agar. Calli still growing after one or two passages on this medium were placed on hormone-free LS medium. At this stage transformants were detected and tested for LpDH activity. pT₂ and pT₇ represent the only two independent experiments that have been performed with the described procedure, and both showed a positive result. Other conditions that were tested but gave negative results differed in incubation time, a lower Ca²⁺ concentration in the post-incubation, and plasmid DNA concentrations (1-50 µg) in the presence and absence of CT-DNA as a carrier. Other procedures were also used, including the poly-L-ornithine method and Ca2+ phosphate-DNA co-precipitation method, which in our hands gave negative results

* These cell lines initially contained LpDH activity, but lost it after 2 months of subculturing.

hydrogenase synthesizes octopine and related compounds such as lysopine in tumour cells induced by an *A. tumefaciens* strain carrying an octopine Ti-plasmid.

The present transformation experiments used octopine Tiplasmid DNA from the wild-type strain Ach5(LBA4001). Plasmid DNA was dissolved at an appropriate concentration in sterile H₂O and its integrity and *SmaI* restriction pattern were checked by agarose gel electrophoresis. The DNA was stored in a sterile Eppendorf tube to which an equal volume of chloroform was added to kill any contaminants. The DNA was kept at 4 °C until use.

The transformation procedure involved incubating protoplasts and DNA in the presence of polyethylene glycol (PEG) and a post-incubation with high Ca2+ concentration (for details see Table 1 legend). This procedure is similar to one used in our laboratory for fusion of protoplasts to produce somatic hybrids. We first determined which PEG concentration would give sufficient cell survival (>20%) and negligible cell fusion during a 30-min incubation. In fusion conditions, PEG is usually present at a 40% concentration for several seconds. In the present experiments we found that a PEG concentration of 13.3% was optimal for an incubation of 30 min; cell survival was 50% and fusion <5%. In our initial DNA transformation experiments we determined whether it was best to start the post-incubation either with a one-step dilution of the PEG by adding 10 ml post-incubation mix containing 125 mM Ca2+ or gradually to dilute the PEG concentration stepwise while increasing the Ca2+ concentration, as is done in fusion experiments. We also examined whether the transformation was dependent on the concentration of Ti-plasmid DNA and the presence of 50 µg calf thymus (CT) DNA as a carrier. Out of 16 experiments, using different conditions, transformants were only found if 10 µg of Ti-plasmid DNA and 50 µg CT-DNA were used during the incubation and if the post-incubation mix was added stepwise. This procedure was tested twice (and both times gave rise to transformants) and Table 1 lists the transformed tissue lines obtained by this procedure in the two independent experiments. No phytohormone-independent calli were obtained in control experiments in which protoplasts were either incubated with CT-DNA alone or without addition of DNA.

From the first successful experiment (plasmid transformation $2=pT_2$), six tissue lines were obtained that showed both phytohormone-independent growth and LpDH activity, but no shoot regeneration. Five other lines proliferated without hormones but had no detectable LpDH activity (Fig. 1). These lines all had the capacity to regenerate shoots; two of them had initially shown the presence of the T-DNA coded enzyme, but had lost enzyme activity after 2 months of subculturing. When shoots that developed from these calli were placed on culture medium, they showed the same properties as regenerants obtained from

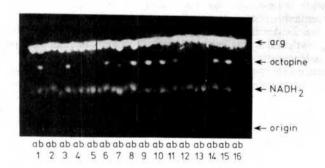


Fig. 3 Electropherogram of the products formed by LpDH activity in tissue extracts. The assay was performed as described by Otten¹². The reaction mixtures contained the extracts of the tissue lines listed in Table 1. a, Channels, reaction mixtures at t=60 min; 1, B6S3 crown gall tumour callus (positive control); 2–13, pT₂ nos 2, 3, 6, 8, 9, 10, 11, 13, 14, 15, 20 and 21, respectively; 14, 15, pT₇ nos 2 and 3; 16, SR₁ shoot (negative control).

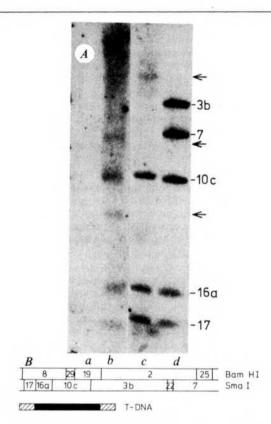


Fig. 4 A, detection of T-DNA in DNA transformed tissue line. The plant DNA was isolated as described by Heyn et al. 13 and as modified by Chilton et al.14. The DNA was digested with the restriction endonuclease SmaI and ar. The DNA was agested with the restriction endouclease Smal and fractionated by agarose (0.7%) gel electrophoresis¹⁰. Blots were prepared according to the procedure of Southern¹⁵ and hybridization was carried out as described by Thomashow¹⁶. A mixture of two recombinant DNA plasmids was used as DNA probe. They consisted of pBR322 in which BamHI fragment 8 (pAL3252) and BamHI fragment 29, 19, 2 and 25 (pAL3076) of the octopine Ti-plasmid were cloned, respectively. The plasmids were separately labelled *in vitro* with ³²P by nick translation¹⁷. The autoradiogram shows the hybridization of the probe to that of the plant DNA. Lane a, SR, (control); lane b, transformant pT₂-13; lane c, transformant pT₂-14; lane d, a one-copy reconstruction of the Ti-plasmid¹⁰. The arrows indicate the border fragments of the T-DNA in the transformants. B, a physical map of a portion of octopine Ti-plasmids representing the restriction endonuclease fragments of BamHI and Smal. The filled bar in the lower part of this figure shows the T-DNA organization as it is found in octopine crown gall tissues

bacterial transformation experiments, that is, an inability to form roots and the absence of apical dominance, resulting in the development of shoots from lateral buds9. Several regenerants were grafted onto stems of N. tabacum var. Samson. Flowers of the mature, grafted shoots possessed an extended stamen and were male-sterile, as was observed previously for transformants9 obtained after bacterial infections of protoplasts (Fig. 2). Another line lost the ability for hormone-independent growth and is now being cultured on medium containing naphthaleneacetic acid and kinetin. It still had LpDH activity. A second independent experiment (PT7) gave rise to two phytohormone-independent, non-regenerating tissue lines with LpDH activity. From the 14 transformed tissue lines obtained in two separate experiments, 11 have been stable from the beginning with regard to their phenotypic properties. The three lines which changed in phenotype have now been stable for at least 9 months. We do not know the reason for the variability in both number of transformants and their phenotype between the two experiments. The transformation event itself, using this procedure, is reproducible, but the transformation frequency and phenotypic diversity apparently differ between experiments. Figure 3 shows the result of the assay for LpDH activity in the various tissue lines.

As further evidence for the transformed nature of the tissues obtained, we have demonstrated the presence of Ti-plasmid

DNA sequences. DNA isolated from two tissue lines (phytohormone independent, LpDH⁺) was digested with the restriction endonuclease SmaI and fractionated by agarose gel electrophoresis. Southern blots were prepared from the gels and hybridized with in vitro labelled DNA probe. Figure 4 gives the results of hybridization for two lines, and shows the contiguous Smal fragments 16a and 10c, which are always found in octopine tumour tissues and which are believed to carry sequences essential for the maintenance of the transformed state of the callus¹⁰; a band corresponding to SmaI fragment 17 is also present, located to the left of 16a; this has only been found in one cell line obtained at our laboratory by in vivo infection. Although both tissue lines analysed contain the same internal Smal fragments, we conclude from the difference in border fragments in which plant DNA and bacterial DNA are joined (indicated by arrows in Fig. 4) that the two lines arose from independent transformation events within one experiment. The presence of Smal fragment 17 in DNA transformants may indicate that frequently a larger part of Ti-plasmid is integrated into the host genome than on infection with the bacterium. Confirmation of this awaits further T-DNA analysis of other transformed tissue lines.

The larger extent of T-DNA in DNA transformants might indicate that the transformation of protoplasts is not due to an A. tumefaciens infection. The following arguments make the possibility of an infection leading to transformation unlikely. The experiments are performed in stringent aseptic conditions. The most conspicuous source of infection would be the plasmid DNA itself if bacteria had survived the isolation procedure. For this reason, the DNA was sterilized with chloroform and sterility was checked by plating a drop of the DNA solution on tryptoneyeast medium. No colonies were formed after 3 days at 29 °C. Moreover, in our experience, in vitro transformation with A. tumefaciens only occurs in certain conditions (F. A. Krens et al., in preparation): the protoplasts must be 3 days old, the incubation time must be >24 h and the density of the bacteria must be at least 3×10^5 per 10^5 protoplasts. None of these conditions is met in the procedure for DNA transformation.

The present results indicate that we have developed a reproducible method for the stable transformation of plant protoplasts with Ti-plasmid DNA. This, for the first time, conclusively demonstrates that the Ti-plasmid is the tumourinducing principle and that A. tumefaciens itself is not a prerequisite for obtaining tumour cells. DNA transformation experiments may aid in elucidating the molecular basis of crown gall tumorigenesis. Experiments have already been started using mutated Ti-plasmids carrying insertions that lead to avirulence of the mutants in vivo. The evidence presented here that plant cells can be stably transformed with DNA will undoubtedly also stimulate programmes for genetic engineering in higher plants.

Received 25 September 1981; accepted 14 January 1982.

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A viral polymerase involved in recognition of influenza virus-infected cells by a cytotoxic T-cell clone

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Influenza virus-specific cytotoxic T lymphocyte (CTLs) can be obtained from mice and humans infected with influenza A viruses^{1,2} and appear to be beneficial in the host response³. The viral target structure recognized by influenza virus-specific CTLs remains a controversial question for two reasons: first, previous specificity analyses were difficult to interpret because of heterogeneous CTL populations, and second, more than one viral protein has been detected serologically on the surface of influenza-infected cells⁴⁻¹⁰. To avoid these problems, antiinfluenza A/PR/8/34 CTL clones were generated and tested for cytolytic activity on target cells infected with recombinant viruses containing known rearrangements of the genes of strains A/PR/8/34 and A/HK/1/68. We report here that one such CTL line lysed target cells only if the infecting virus possessed the polymerase gene P3 of A/PR/8/34. This suggests that the P3 gene product either induces a virus-specific cell-surface antigenic modification or is expressed on the surface of the infected cell, and hence is involved in recognition of target cells by the anti-influenza CTL line.

Influenza type A-specific CTLs were generated as described elsewhere 11,12. BALB/c mice were inoculated intraperitoneally (i.p.) with A/PR/8/34; 2-4 weeks later, the primed spleen cells were cultured in vitro for 10 days in the presence of PR8infected BALB/c spleen cells. Our method differs from the standard technique in that the culture from which the CTL line described here (BCIP3.1) was derived was then treated with monoclonal anti-Lyt 2 antibody 3-168.8 (ref. 13) (the gift of F. Fitch, University of Chicago) plus complement. Initially this treatment was performed to enrich for helper T cells. Although the percentage of Lyt 2⁺ cells was reduced at the time of treatment to less than 1% (as shown by immunofluorescence), most of the cultures retained antiviral CTL activity and ~5-10% were 'strain-specific'. CTL clones derived from these cultures have subsequently been shown to be Lyt 2+ by immunofluorescence. 'Cross-reactive' CTL lines alone were isolated from cultures not treated with anti-Lyt 2 antibodies. All cultures were propagated in vitro in medium containing 20% supernatant from concanavalin A (Con A)-stimulated rat spleen cell cultures and, after 2-3 months, cloned on a feeder layer of irradiated BALB/c peritoneal exudate cells. The CTL line BCIP3.1 described here has been maintained in culture for 6

The specificity of the BCIP3.1 line was examined in microcytotoxicity assays on P815 cells infected with various influenza viruses. As shown in Table 1, the cytotoxic activity was restricted to cells infected with influenza viruses A/Swine/31 (A/SW/31), A/Puerto Rico/8/34 (A/PR/8/34), A/Melbourne/35 (A/Mel/35) and A/BH/35. The failure of BCIP3.1 to lyse target cells infected with the other influenza A viruses was not due to inadequate expression of viral antigens on these target cells as they were killed by one or several other CTL lines included as positive controls (J.R.B., in preparation).

As the BCIP3.1 clone lysed cells infected with A/Puerto Rico/8/34 (PR8) but not with A/Hong Kong/1/68 (HK) (Table 1), it was possible to examine its specificity further using target cells infected with recombinant viruses containing various

arrangements of parental PR8 and HK genes. Due to the segmented genome of influenza viruses, such recombinant viruses can be readily produced by mixed infection, and their genotype determined by RNA-gel electrophoresis¹⁴. Thus, the BCIP3.1 line was tested for cytotoxic activity against P815 cells infected with 17 different PR8-HK recombinant viruses of defined genotypes (gifts of Drs P. Palese, J. Schulman and M. Lubeck, Mt Sinai School of Medicine). It can be seen from the top half of Table 2 that the BCIP3.1 clone does not lyse targets infected with 10 recombinant viruses which lack the PR8 P3 gene. Particularly striking is the recombinant E61-24-P15 which differs from PR8 solely in the substitution of the P3 gene. This absence of cytotoxicity is due neither to the failure of these viruses to infect the target cells, nor to a general resistance of the infected cells to lysis by CTLs, as the cells were lysed to the same extent as PR8-infected cells by cloned CTL lines specific for other viral target structures (J.R.B., in preparation). In contrast, the bottom half of Table 2 shows that target cells infected with recombinant viruses having the PR8 P3 gene were lysed. As PR8 P3 is the only gene in the recombinant virus panel which correlates absolutely with the presence or absence of cytotoxic activity, we conclude that the BCIP3.1 T-cell clone recognizes a target structure on P815-infected cells which requires the presence of the PR8-derived P3 gene.

However, lysis of recombinant virus-infected target cells by the BCIP3.1 clone is not a simple all-or-none phenomenon because the extent of lysis seems to be affected to some extent by additional genes: for example, the highest ⁵¹Cr release (45–60%) was observed for the four recombinants E61-24-H16, J1, 13B and J5 which have in common the PR8-derived genes P3 (prerequisite for lysis), P2 and Np. However, substitution of either the P2 or nucleoprotein (NP) genes of PR8 by the corresponding HK genes resulted consistently in reduced ⁵¹Cr

Table 1 Cytotoxic activity of BCIP3.1 CTL line on P815 cells infected with various influenza virus strains

Virus strain (subtype)			% Specific ⁵¹ Cr release
A/Swine/31)		27
A/WSN/33			3
A/Puerto Rico/8/34			37
A/Melbourne/35	1		31
A/BH/35	i		17
A/Hickox/40	l		2
A/Bellamy/42	> H1N1		1
A/Weiss/43	1		5
A/Cameron/46			0
A/Fort Monmouth/1/47	'	•	0
A/Forth Worth/50			2
A/Malaya/54	İ		0
A/Denver/57)	_	0
A/Ann Arbor/57	H2N2	•	0
A/Japan/305/57	H2N2		0
A/Hong Kong/1/68	H3N2		0
A/Victoria/68	H3N2		0

Type A influenza viruses have been grouped into three subtypes designated H1N1, H2N2 and H3N2. H1, H2 and H3, and N1, N2 each designate serologically non-cross-reactive serotypes of the haemagglutinin (HA) and neuraminidase (NA) molecules, respectively. Each subtype consists of many distinct but antigenically related glycoproteins that characterize individual virus strains. Infectious allantoic fluid of these viruses was used to infect P815 cells as described previously Six hours after infection, cells were incubated for 1 h with ⁵¹Cr at 37 °C washed twice and then dispensed into wells of microtitre plates (104 cells per well). BCIP3.1 cell ratio of 9:1. The cells were pelleted lightly and after 4 h incubation at 37 °C, one-half of the culture supernatant was removed for determination of the concentration of released by y counting. Per cent specific release was determined according to the equation: experimental release (in the presence of CTLs)spontaneous release (no CTLs)/total release (in the presence of detergent)-spontaneous release. Each assay was 'performed in triplicate.

release, that is, 23-26% and 13% for recombinants E61-13-H17, E61-13-H20 and E61-13-H19 respectively.

The frequency of CTLs possessing the BCIP3.1 specificity in the influenza immune response is unknown. Previous frequency analyses could have placed this specificity with either 'strainspecific' or 'cross-reactive' CTLs, depending on the viruses tested. The finding of at least two other clones, one having an apparently identical specificity and another with a slightly different specificity but correlating with the polymerase gene complex (unpublished data) suggests that the BCIP3.1 is not an unusual clone. The role and relative importance of the polymerase gene complex in the CTL immune response are unknown.

The association of the lytic activity of BCIP3.1 with the presence of the PR8 P3 gene can be explained by two mechanisms. First, BCIP3.1 could recognize the P3 protein itself. Although P3 is present in infected cells in only small quantities compared with the other viral proteins, and appears to be located predominantly in the nucleus and cytoplasm 13,16, it remains a possible target structure, as the amount of viral antigen needed for lysis by CTLs is unknown and the possible presence of small amounts of cell-surface-associated P3 has not been investigated. Alternatively, P3 may be required for the expression of the target structure recognized by BCIP3.1. In this respect, note that the function of the P3 protein in influenza virus infection is incompletely understood. Results obtained for temperature-sensitive mutants indicate a role for P3 in complementary (messenger) RNA synthesis¹⁷, and suggest that P3 is involved in the transport of the viral transcription-replication complex (consisting of the P1, P2, P3 and NP proteins¹⁶) from nucleus to cytoplasm¹⁸. Thus the PR8 P3 protein may modify another viral (or cellular) gene product recognized by the BCIP3.1 line, or may be responsible for transport of this antigen to the cell surface. The finding that the cytotoxic activity of BCIP3.1 is also related to the presence of two other proteins (P2 and NP) known to be involved in the virus transcriptionreplication complex, does not help to distinguish between these possibilities as this observation could reflect either the involvement of P2 and NP in the surface expression of P3 or the higher efficiency of a PR8 P2-P3-NP complex in the expression of the actual antigen recognized by BCIP3.1.

Table 2 Cytotoxic activity of BCIP3.1 CTL line on P815 cells infected with various recombinant influenza viruses

				Gene					% Specific
	P1	P2	P3	HA	NA	NP	M	NS	51Cr
Virus									- release
PR8	P	Ρ,	P	P	P	P	P	P	21-47
HK	H	H	Η	Η	Η	Η	Η	H	2-7
E61-24-P15	P	P	H	P	P	P	P	P	1
E61-13-P15	H	H	Η	P	P	P	P	P	0
E61-24-P11	P	Ą,	Η	P	P	P	P	Η	0
P37	H	P	Н	P	Н	P	P	P	0
E61-13-H15	H	P	Н	Η	Η	Η	P	H	1
E61-13-P19	H	H	H	P	P	Η	Η	P	0-4
3385	H	H	H	H	Η	P	P	P	4
3376	H	H	Η	P	Η	Η	P	H	2
E61	H	Η	Н	P	P	H	H	Η	1
P50	H	H	Η	P	Н	H	H	H	6
E61-24-H16	Н	P	P	H	Н	P	P	Н	60
J1	P	P	P	H	P	P	P	P	47
13B	P	P	P	H	P	P	H	P	45
J5	H	P	P	H	P	P	H	H	51
E61-13-H17	P	P	P	H	H	H	P	P	26
E61-13-H20	H	P	P	H	H	H	P	P	23
E61-13-H19	H	H	P	H	Н	P	H	P	13

⁵¹Cr-release assays were performed on P815 cells 7 h after infection with recombinant viruses containing various combinations of PR8 (P) and HK (H) genes coding for the three viral polymerases (P1, P2 and P3), haemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix (M) and non-structural (NS) proteins. The pooled data from three separate cytotoxicity assays are shown.

In conclusion, we have demonstrated that the PR8 P3 gene product, perhaps in association with other viral gene products, is involved in the recognition of target cells by an anti-influenza CTL line. However, it remains to be determined how P3 affects the target cell surface and whether it plays an important part in the recognition of influenza-infected target cells by CTLs.

We thank S. Fazekas de St Groth and F. Melchers for their critical reading of the manuscript and L. Forni for the Lyt 2 immunofluorescence testing. This work was supported in part by National Institute of Allergy and Infectious Diseases grant A113989 and National Multiple Schlerosis grant RG-851C6. The Basel Institute for Immunology is supported by Hoffman-La Roche & Co.

Received 29 October 1981, accepted 15 January 1982.

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Monoclonal antibody to Lyt 2 antigen blocks H-2I- and H-2Kspecific mouse cytotoxic T cells

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It has been proposed that the murine cell surface antigen Lyt 2 could serve to distinguish cytotoxic T lymphocytes (CTLs) and their immediate precursors, which bear Lyt 2, from helper T cells, which do not1. Later work, however, has demonstrated that Lyt 2+ cells can 'help' B cells mature into antibody-secreting cells, provided that the B cells differ from the allo-helper T cells at the K or D region of the H-2 major histocompatibility complex (MHC)2. Furthermore, a T-cell line has recently been described which is Lyt 2⁻ but specifically cytotoxic for *H-21* region determinants^{3,4}. These new findings have prompted an alternative hypothesis, that Lyt 2 antigen does not discriminate between cytotoxic and non-cytotoxic (helper) T cells, but rather between I-region- and K/D-region-specific cells⁵. In this new view, the apparent association between presence of Lyt 2 antigen and killer T-cell function merely reflects the tendency, by no means absolute 6-8, for CTLs to recognize K- or D- rather than I-region determinants⁹. We show here that I-regionspecific murine CTLs, generated in primary in vitro culture, do in fact bear the Lyt 2 antigen, as do CTLs directed against the K region of H-2.

Our method is based on the observation that the lytic reaction between mouse CTL effectors and their targets can be blocked by addition of anti-Lyt 2 antibody directly to the cytotoxicity assay¹⁰⁻¹³. Although it is not clear how the antibody interferes with target cell lysis, the finding that inhibition does not require Lyt 2 expression by the target cells strongly suggests that recognition of the Lyt 2 molecules on the CTL effector cell is

Table 1 Cytolysis by I-region-specific CTLs is inhibited by monoclonal antibody to Lyt 2

	Line	Responder	Stimulator	Target	Antigen	Antibody added	% Specific lys (s e m.)
Expt A							
	1	AQR	T6R	T6R	$\mathbf{I}_{\mathbf{d}}$	None	66 (2)
	2	AQR	T6R	T6R	Iq	Antı-Lyt 2	3 (2)
	3	AQR	A	Α	K ^k	None	53 (3)
	4	AQR	Α	Α	K ^k	Antı-Lyt 2	0(1)
Expt B						•	• • •
	5	AQR	T6R	T6R	I _d	None	26 (3)
	6	AQR	T6R	T6R	I.a I.a	Anti-Lyt 2	4 (1)
	7	AQR	A	A	K' ^k	None	14 (3)
	8	AQR	Ā	A	Kk	Anti-Lyt 2	2(1)
	9	T6R	AQR	AQR	Ĭ ^k	None	11 (1)
	10	T6R	AQR	AQR	K ^k K ^k I ^k I ^k	Antı-Lyt 2	3 (1)
Exapt C				-	-	,	- (-,
	11	AQR	T6R	T6R	I _d	None	34 (3)
	12	AQR	T6R	T6R	Ιq	Antı-Lyt 2	12 (1)
	13	AQR	T6R	T6R	I_{σ}	Anti-Lyt 2.2	9(1)
	14	AQR	T6R	T6R	Ια	Nude NMS	33 (3)
	15	AQR	T6R	T6R	Ιq	Anti-Lyt 1	50 (4)
	16	AQR	T6R	AQR	None	None	2(1)
	17	AQR	A	Ā	K*	None	48 (5)
	18	AQR	Â	Ä	K ^k	Antı-Lyt 2	5 (2)
	19	T6R	AQR	AQR	$\overline{I^k}$	None	28 (3)
	20	T6R	AQR	AQR	Ϊ ^k υ	Antı-Lyt 2	7(1)
	21	T6R	AQR	T6R	None	None	2(1)

Spleen cells from unprimed responder mice were mixed with irradiated (1,500 rad) stimulator spleen cells, and cultured in conical-bottomed microtitre wells such that each well contained 3×10^5 responder cells and 5×10^5 stimulators in 0.2 ml of RPMI-1640 medium with 10% fetal calf serum, 10 mM HEPES, antibiotics and added glutamine. After 5 day culture at 37 °C, the medium was shaken off and the cell pellet resuspended in 0.1 ml of serum-supplemented medium containing the antibody to be tested for inhibitory effect. Another 0.1 ml of medium was then added containing 2×10^4 51Cr-labelled concanavalin A-stimulated spleen cells of the indicated strain, as targets. After a 4 h incubation at 37 °C, the plates were centrifuged at 1,000 r.p m for 10 min and 0.1 ml of supernatant was removed for counting Per cent specific lysis was calculated as $100 \times (\text{experimental} - \text{low control})/(\text{acid lysis} - \text{low control})$. Six to elght statistically independent replicates were performed for each experimental condition tested; the equivalent ratio of viable effector cells to targets is $\sim 10:1$. Anti-Lyt 2 is hybridoma clone 53-6.7 of ref. 15, used at 1/100 in expt A, and 1/500 in expts B and C. Normal nude mouse serum (nude NMS) and anti-Lyt 1 antibody (clone 53-7.3) were used at 1/500 Ascites fluid containing mouse monoclonal antibody to Lyt 2.2 was used at 1/100. Strain abbreviations and genotypes are as follows: AQR, B10.AQR($K^4I^{k/d}D^d$); T6R, B10.T(6R) ($K^4I^{k/d}D^d$); A, B10.A($K^kI^{k/d}D^d$). All three strains are TD^a

involved^{10,11}. Cytotoxicity can be blocked by conventional alloantisera and by monoclonal antibodies specific for the Lyt 2 molecule^{10–13}.

To examine I-region-specific CTL responses of unprimed mice, which have been reported to be considerably weaker than the in vitro responses obtained from in vivo immunized responders6, we performed the 5 day in vitro sensitization culture and the 4 h 51Cr-release assay for cytotoxicity in conical-bottomed microtitre wells, which we have found produce satisfactory responses to several 'weak' antigens (that is, non-H-2 determinants). To generate I-region immune CTL effectors, we carried out reciprocal in vitro sensitizations between congeneicresistant mouse strains B10.AQR and B10.T(6R), which differ only at the I region of the MHC14. B10.A stimulators, which differ from B10.AQR only at the K region, were used to produce K-specific CTLs as a positive control. At the end of the 5 day sensitization period, the CTL assay was carried out in the presence of either monoclonal antibody to Lyt 2 or control serum. A monoclonal rat antibody to a nonpolymorphic portion of the murine Lyt 2 molecule was used (clone 53-6.7 of ref. 15), and we found that this antiserum inhibits both I- and K-regionspecific CTL reactions at dilutions >1/3,200.

Table 1 shows the results of three consecutive experiments; the monoclonal antibody to Lyt 2 not only blocks K-specific CTLs as expected, but also interferes with I-region-specific CTLs, and to the same extent. CTLs specific for Iq (Table 1, lines 2, 6, 12), I' (Table 1, lines 10, 20) and K' (Table 1, lines 4, 8, 18) are all strongly inhibited by the addition of anti-Lyt 2 antibody. The specificity controls show that the CTLs are in fact I-region specific (compare lines 11, 16 and 19, 21), as they kill only those targets which present the appropriate alloantigen; target cell death thus cannot be attributed to an antigen-nonspecific cytolytic event. Because the source of monoclonal antibody was serum from nude mice bearing the hybridoma as a subcutaneous tumour, controls were carried out (experiment C of Table 1) to show that the inhibitory activity was attributable to the anti-Lyt 2 antibody in the serum, rather than to some unsuspected material which might be present in sera from tumour-bearing nude mice. In studies involving conventional (non-monoclonal) antibodies of the Lyt type, the best control¹⁶ is to compare the

effect of the antibody on responder mice which differ only at the locus in question (here Lyt 2), but such a control is impossible in our study because it would require mice that are congeneic at both the Lyt 2 and I-region loci. Table 1 shows that neither normal nude mouse serum (Table 1, line 14) nor serum from nudes carrying a hybridoma (clone 53-7.3 of ref. 15), which secretes antibody to mouse Lyt 1 antigen (Table 1, line 15), was able to inhibit CTL effector activity, showing that the inhibitory activity of the anti-Lyt 2 hybridoma antiserum depended on the presence of antibodies to Lyt 2. Furthermore, line 13 in Table 1 shows that strong inhibition of cytotoxicity can also be obtained by a second monoclonal anti-Lyt 2 antiserum (provided by Dr U. Hammerling), which is specific for the polymorphic determinant Lyt 2.2 and is used as ascitic fluid from tumour-bearing mice. As inhibitory activity is found only in those sera from hybridoma-bearing nude mice which include anti-Lyt 2 antibody, and both monoclonal reagents (reactive with different determinants on the Lyt 2 molecule) exhibit potent blocking activity, we are confident that the inhibition seen results from an interaction between anti-Lyt 2 antibody and CTL surface Lyt 2 molecules. In summary, Table 1 shows that CTL activity against both I- and K-region antigenic determinants can be blocked by monoclonal anti-Lyt 2 antibodies, and that this blocking is attributable to the anti-Lyt 2 antibody in such reagents.

Although inhibition of lysis by anti-Lyt 2 antibody, as argued above ^{10,11}, need not involve Lyt 2-bearing target cells, we considered whether the inhibition shown in Table 1 might involve, not an interaction between the antibody and the CTL effectors, but rather a previously unknown protective effect of anti-Lyt 2 antibody on the concanavalin A blast targets, a portion of which exhibit Lyt 2 antigen¹². The experiment shown in Table 2, however, shows that strong inhibition of cytotoxicity by anti-Lyt 2 antibody is obtained even when the target cells are lipopolysaccharide activated B cells, selected by adherence to plastic plates coated with anti-mouse immunoglobulin; these cells do not carry surface Lyt 2. Thus, inhibition of *I*-region-specific CTLs can be induced by an interaction between anti-Lyt 2 antibody and the CTL effector cell itself.

It is not yet clear at what stage of cell-mediated cytotoxicity the Lyt 2-mediated blocking is achieved, although the

Table 2 Inhibition of I-region-specific CTLs by anti-Lyt 2-B cell blast targets

Responder	Stimulator	Antibody added	% Specific lysu (s.e.m.)
AQR	T6R	None	45 (2)
=		Antı-Lyt 2	17 (5)
		Anti-Lyt 1	41 (3)
		Nude NMS	43 (2)
T6R	AOR	None	54 (4)
	_	Antı-Lyt 2	28 (3)
		Antı-Lyt 1	52 (4)
		Nude NMS	45 (3)

Experimental conditions were as for Table 1, except that the ⁵¹Cr-labelled targets were plate-selected ²⁶ B cells stimulated with lipopolysaccharide (5 µg ml⁻¹) for 48 h before use. All inhibitory antibodies were used at 1/300 dilution Per cent lysis is presented as mean (and s.e.m.) of 16 wells per group.

tantalizing proximity of the Lyt 2 gene to the mouse V_{κ} locus¹⁷, which controls the variable region of the κ light chain, and the demonstration¹³ that anti-Lyt 2 antibody blocks conjugate formation, but not lysis itself, suggest that inhibition of specific target recognition may be involved. In any case, our results indicate that however the Lyt 2-bearing molecules in the effector cells are involved in cytolysis, they are likely to have an equivalent role regardless of the genetic region—I or K/Dwhich controls the target cell antigens being recognized by the effector CTL. They also demonstrate, a fortiori, that I-specific CTLs induced by primary in vitro sensitization must be Lyt 2+ cells.

We cannot exclude the possibility that some I-specific CTLs produced from normal spleen cells might be Lyt 2-, and thus resemble the Lyt 2⁻, I-A^k-specific cytolytic T-cell line recently described^{3,4}, but we believe that most *I*-specific CTLs, like their more easily generated K/D-specific counterparts¹ and many cytolytic T-cell lines^{18,19}, are probably Lyt 2⁺, based on the unambiguous inhibition of cytotoxicity produced by the anti-Lyt 2 reagents in the present experiments. Our results are consistent with a recent study²⁰ showing that *I*-specific primary CTL effectors could be killed by sufficiently potent anti-Lyt 2 antisera and complement.

Generation of cytotoxic effector cells is now believed to involve collaboration between the CTL precursors and a 'helper' T cell which produces the helper factor interleukin-2 (IL-2)^{21,22}. Although K-region-stimulated secretion of IL-2 by Lyt 1⁻²⁺ cells has been reported recently^{23,24}, we have found²⁵ that positively selected Lyt 2+ cells produce much less IL-2, even in response to K-region disparities, than do I-regionstimulated Lyt 2 cells. We thus favour a model for CTL generation across MHC incompatibilities in which most of the IL-2-producing helper cells are I-region sensitive and Lyt 2 and most of the CTLs, whether K- or I-region responsive, are nonetheless Lyt 2+. The extent to which the allospecific CTLs resemble CTLs generated in vivo or to 'foreign' antigens in syngeneic contexts requires further investigation.

We thank Ms F. Lebow for technical help, Dr U. Hammerling for antibody, Dr C. David for mice, and Ms L. Stevenson and Ms M. King for preparing the manuscript. This work was supported by NIH grants AG-02497 and AG-02152. R.A.M. was a Special Fellow of the Leukemia Society of America.

Received 28 August 1981; accepted 15 January 1982.

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Glycolipid affinity purification of migration inhibitory factor

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Migration inhibitory factor (MIF) is an immune mediator secreted by primed lymphocytes on in vitro challenge by antigen or mitogen, which elicits various physiological responses from its target cell, the macrophage^{1,2}. Previous studies suggest that membrane glycoconjugates have an important role in regulating the interaction between MIF and the macrophage. A macrophage glycolipid functions as a putative receptor for MIF as evidenced by its ability to enhance the macrophage response to MIF^{3,4}. This enhancing ability of glycolipids requires the presence of sialic acid⁵ and fucose⁶; these carbohydrate residues are also required on the macrophage surface for the induction of an intact response by MIF5,7. Furthermore, it has been reported4 that incubating a preparation of mixed gangliosides from bovine brain with lymphocyte supernatants abolished their MIF and macrophage activating factor (MAF) activities. Taken together, these results are evidence that a glycolipid serves as a membrane receptor for MIF. We predict that glycolipids which enhance the macrophage response to MIF, also bind MIF with subsequent elution of biological activity, and present here evidence in support of this notion. We also demonstrate that glycolipid covalently bound to agarose provides a novel means of affinity purification of MIF.

Guinea pig⁸ and human⁹ MIF have been fractionated in this laboratory by various conventional techniques including gel filtration, electrophoresis and isoelectrofocusing. These procedures separate macromolecules on the basis of their physicochemical properties and thus are limited by their lack of specificity. Affinity chromatography is a useful technique for the purification of many proteins, and has been used successfully to purify MIF^{10,11}. Here we used an insoluble agarose support, derivatized with a macromolecular arm (poly-L-lysine¹²) followed by covalent attachment of gangliosides to this support¹³, to purify guinea pig MIF.

Results of initial purification experiments are shown in Fig. 1. MIF does not bind or is not adsorbed to columns containing unsubstituted agarose in the presence of phosphate-buffered saline (PBS) (0.15 M NaCl in 0.01 M phosphate-buffer pH 7.4). However, a substantial amount of MIF activity does adsorb to agarose covalently bound to a mixed preparation of bovine brain gangliosides. This adsorption appears to be specific as no adsorption occurs on agarose substituted with other preparations of glycolipids, for example, disialoganglioside (GD_{1a}), bovine brain grey matter and guinea pig brain (Fig. 1). Furthermore, the adsorbed MIF activity was recovered by elution with 0.2 M fucose in 1 M potassium thiocyanate (KSCN). Recovery of active MIF showed that use of ganglioside-agarose to purify MIF was possible.

In the experimental protocol described in Fig. 1 legend, a significant amount of protein (12-30%) eluted with MIF (data

Table 1 Ability of glycolipids to enhance MIF response

Macrophages preincubated with:	M199	Bovine brain mixed gangliosides	% Inhibition of migration Guinea pig macrophage glycolipid	Bovine brain grey matter glycolipid	Guinea pig brain glycolipid
Expt				3,	giyeenpid
1	6	37			
2	3	38		1	
3	6	30		3	
4	20	48		0	
5	7	11 55492	36	U	
6	28		43		22
7	24		9.70		24
8	24		35 52		17

Glycolipid preparations were tested for their ability to enhance the macrophage response to MIF, using methods described elsewhere³. Briefly, the test glycolipid deposited with lecithin and cholesterol as a thin film on a glass wall *in vacuo*. Liposomes were formed under nitrogen in buffer by agitation and sonication. After centrifugation the small liposomes were incubated with macrophages at 37 °C for 1 h. Cells were then washed and assayed for their response to a low dose of MIF in a capillary tube assay. The MIF response is shown as % inhibition of migration. The preparation of mixed gangliosides from bovine brain and glycolipids from guinea pig macrophages were the only preparations able to enhance the macrophage response to MIF.

not shown). Experiments were done to investigate whether different buffer conditions would remove unwanted protein from the eluent (Fig. 2). MIF was allowed to bind in a buffer of relatively high salt concentration (0.3 M NaCl in 0.01 M phosphate buffer pH 7.4 [0.3 M PBS]). The column was then washed with 0.6 M NaCl in 0.01 M phosphate buffer pH 7.4 (0.6 M PBS) and MIF eluted with 1.0 M KSCN in 0.01 M phosphate buffer pH 7.4 (1.0 M PBS). Most of the protein eluted before MIF (Fig. 2), thereby facilitating a significant increase in the specific activity of MIF (Table 2).

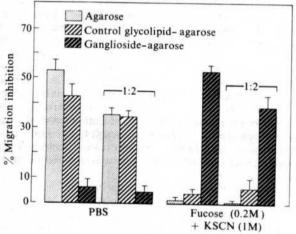


Fig. 1 Specific binding of MIF to ganglioside-agarose columns. Guinea pig MIF was prepared from culture supernatants of concanavalin A-stimulated lymph node cells from PPD-sensitized Hartley guinea pigs and chromatographed on Sephadex G-100 columns. One unit of MIF was defined as the greatest dilution giving 20% inhibition of migration in the capillary tube assay5. To determine the number of MIF units in a particular fraction, serial twofold dilutions were assayed. Sepharose 4B (Pharmacia) was activated by CNBr and coupled to poly-L-lysine (molecular weight 500,000; Sigma)12 Mixed gangliosides from bovine brain (Supelco) were coupled to poly-L-lysine-agarose using carbodiimide¹³. Approximately 80% of the Approximately 80% gangliosides were insolubilized as determined by the amount of free sphingosine in the reaction mixture. The coupled agarose was diluted fivefold with Sepharose 4B and packed in glass columns $(1 \times 10 \text{ cm})$ with a total bed volume of 3-4 ml. MIF $(16-20 \text{ units or } \sim 10 \text{ ml})$ equivalents of original supernatant) was percolated on to the column at room temperature with a flow rate not exceeding 10 ml h-1. The column was equilibrated and washed with PBS buffer followed by 0.2 M fucose in 1 M KSCN; 10 ml of each wash were collected on ice, vacuum-concentrated to 3 ml and dialysed overnight against minimal essential medium plus Earle's salts (Microbiological Associates). The equilibration and elution buffers and the types of agarose used are indicated. B represents unsubstituted agarose (poly-L-lysine-agarose without ganglioside);
represents agarose coupled to glycolipids prepared from bovine brain grey matter6, guinea pig whole brain6 or GD1a; represents agarose coupled to bovine brain mixed gangliosides (BGSL). Note that MIF activity (undiluted and 1:2 dilution) elutes through the control columns with the PBS wash and through the BGSL-agarose column only with the fucose-KSCN buffer. The differences between all six experiments were statistically significant (P < 0.001) by Student's t-test.

The results shown in Table 1 indicate a correlation between the reversible binding activity of a glycolipid preparation and its ability to enhance the macrophage response to MIF. We succeeded in enhancing MIF activity by preincubating macrophages with their putative glycolipid receptors for MIF as described previously⁶. The only glycolipid preparation to which MIF could be bound and recovered (mixed ganglioside from bovine brain) was also able to enhance the macrophage response to MIF—further evidence that gangliosides serve as MIF receptors.

As carbohydrate is known to be an essential part of the macrophage response to MIF⁵⁻⁷ we investigated the use of fucose as a specific eluent in a more effective purification method for MIF. Preliminary studies indicate that fucose alone is unable to elute MIF from ganglioside-agarose columns. KSCN alone was effective in the recovery of MIF, which is not surprising in view of what is known about carbohydrate-protein interactions. Simple monosaccharides, such as fucose, exhibit biological effects only at very high concentrations^{7,10,14}. In the case of ganglioside receptors, it appears that the specific oligosaccharide sequence and not just the presence of sialic acid is important for receptor function 15,16. It may be necessary to elute MIF with the oligosaccharide moiety of the putative glycolipid receptor. This could also explain why the ganglioside-agarose matrix is still effective for the purification of MIF even though the gangliosides are covalently bound to the polylysyl backbone

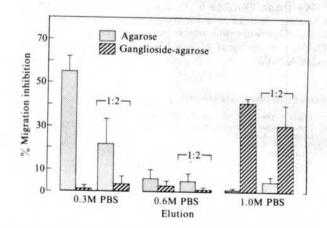


Fig. 2 Purification of MIF by salt elution from ganglioside-agarose columns. Partially purified MIF was subjected to affinity chromatography on ganglioside-agarose columns as described in Fig. 1 legend with the following modification. The equilibration and two sequential buffer elutions consisted of 0.01 M phosphate buffer pH 7.4 with 0.3 M NaCl, 0.6 M NaCl, and 1.0 M KSCN, respectively. represents unsubstituted agarose; ☑ represent agarose coupled to bovine brain mixed ganglioside. Note that MIF activity could be quantitatively recovered in the 1.0 M KSCN elution after binding in 0.3 M NaCl buffer. In three experiments, differences between each of the elutions were statistically significant (P < 0.001).

Table 2 Affinity purification of MIF on ganglioside-agarose

	Total protein (mg)	Total units	Specific activity (U mg ⁻¹)	Purification
Before purification	0.20	16	80	1
0.3 M	0.061	0	0	0
0.6 M	0.016	0	0	0
1.0 M	0.0017 (3%)	13.4 (84%)	1,914	24

The data summarize an experiment described in Fig. 2 legend. Percentage yield is given in parentheses. The breakthrough is represented by 0.3 M PBS, elution 1 is represented by 0.6 M PBS and elution 2, 1.0 M PBS. Purification may be more or less marked depending on the MIF preparation. One unit of MIF activity was defined as the highest dilution giving 20% inhibition of migration in the capillary tube assay.

through their sialic acid residues. MIF may recognize the oligosaccharide and not just sialic acid.

However, the method used here for coupling gangliosides to agarose does present the possibility that hydrophobic forces are involved in the ganglioside-MIF interaction, together with an affinity of MIF for carbohydrate residues. The dual nature of interactions between macromolecules has been shown previously for interferon¹⁴ and tetanus toxin¹⁷. Furthermore, the increasing ionic strengths of the buffers used in the protocol described in Fig. 2 legend may strengthen the hydrophobic interactions. This would result in a high binding affinity necessitating the use of chaotropic ions, such as thiocyanate in the case of MIF and serum growth factor¹⁸ or 7 M guanidine HCl in the case of cholera toxin¹³, which promote macromolecular unfolding and dissociation.

It is probable that only a minor unknown component of the bovine brain ganglioside mixture has a biospecific affinity for MIF⁴. We therefore have to consider the overlapping effects of other ganglioside-protein interactions which obscure the particular interaction in question. It might be better to examine an affinity adsorbent that contains only the homogeneous putative glycolipid receptor for MIF19

We have shown that gangliosides covalently bound to derivatized agarose provide a powerful tool for the purification of MIF by solid phase affinity chromatography. Furthermore, it may provide a means of separating biochemically similar lymphocyte mediators. Work is being done to define further the nature of the binding between the gangliosides and MIF components.

We thank Caroline S. F. Yu for technical assistance and Christine Sleiman and Ramona Gonski for secretarial assistance. This work was supported by N.I.H. grant AI-O7685. D.Y.L is a recipient of a N.I.H. Young Investigator Award AI/CA 16021.

Received 16 November; accepted 24 December 1981

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Persistence of axial orientation cues in regenerating intima of cultured aortic explants

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The endothelial cells of arteries have a preferential orientation parallel to the axis of blood flow¹. This axial orientation is maintained even at the interface between two branching or anastomosing vessels1, and is re-established by newly regenerated endothelial cells in regions of intimal wounds2. The cause of the orientation, however, remains unclear. Two possibilities have been proposed, shear stress from blood flow³ and 'contact guidance' by extracellular matrix', but experiments to distinguish between them have been limited by the inaccessibility of the intima to systematic manipulation in vivo. and by the failure of organ-cultured vessels to demonstrate intimal regeneration comparable with that observed in vivo 5.6. We have recently developed a technique for the culture of whole rat aorta explants which provides easy access to the intimal surface in vitro7. Additionally, our technique provides the first organ culture system in which the regeneration of endothelium in response to wounds performed in vitro has many of the characteristics of such regeneration in vivo. By studying the migration of regenerating endothelial cells into intimal wounds produced in vitro, we have obtained new evidence in favour of the extracellular matrix as the short-term determinant of endothelial cell orientation. Moreover, although this apparent effect of the matrix can be modified temporarily by suitable wounding procedures, axial orientation cues persist even in the absence of blood flow.

Small pieces of rat aorta, pinned with glass needles to a Sylgard-coated culture dish and incubated in Medium 199 (supplemented with 30% heat-inactivated calf serum), maintain an intact endothelium, as determined by scanning and transmission electron microscopy, for as long as 10 days in culture (R.W.J., S. K. Anderson and J. D. Sheridan, in preparation). The endothelial cells respond to in vitro intimal wounds by migration and mitosis near the wound edge. A striking result is that the orientation of the regenerating cells depends on the type of wound produced.

With mechanical denudation it is theoretically possible to make four different wounds defined by the orientation of the wound edge and the direction of the mechanical stress used to make the wound. The edge of the wound can be oriented either circumferentially, as occurs in most experimental in vivo wounding procedures, or axially. The wound can be created by applying mechanical stress in the same direction as the edge or perpendicular to the edge. Thus, the following wounds are defined: (1) circumferential wounds made axially (the most common in vivo type³; see Fig. 1a); (2) circumferential wounds made circumferentially (not previously described; see Fig. 1b); (3) axial wounds made axially (related to the 'narrow scratch' wound recently reported⁸; see Fig. 1c); and (4) axial wounds made circumferentially (not previously described; see Fig. 1d). We have made all four types of wound in our aorta cultures and have monitored the endothelial regeneration for several days thereafter.

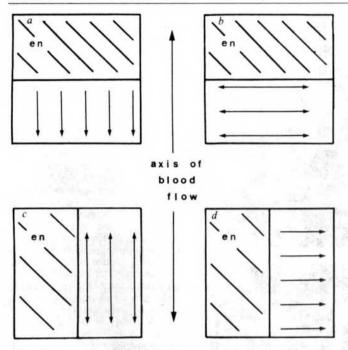
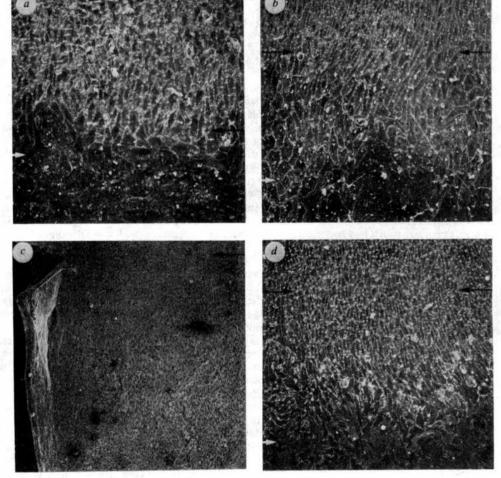


Fig. 1 Diagram of wounds made on cultured explants: a, circumferential wound edge made by wounding axially; b, circumferential wound edge made by wounding circumferentially; c, axial wound edge made by wounding axially; d, axial wound edge made by wounding circumferentially. Wounds are made with a rubber policeman at the 4th day in vitro. Unmarked arrows indicate the movement of the rubber policeman during wounding. Hatched area (en) indicates the endothelium remaining after wounding.

Figure 2a-c shows the results of regeneration from a circumferential wound made axially. After only 24 h of recovery (Fig. 2a) the endothelial cells have become elongated in an axial direction and have begun to migrate on to the denuded surface. These processes are even more apparent at 48 h after wounding (Fig. 2b). By 3 or 4 days, depending on the amount of surface removed originally, the endothelial cells completely resurface the explant (Fig. 2c). Figure 2d shows a circumferential wound made circumferentially. The preparation was fixed 48 h after wounding. It is apparent that orientation of the endothelial cells on the resurfaced area is almost exclusively axial. However, at the migrating front there is a greater degree of endothelial flattening and a more random endothelial orientation than when the wound is made axially.

When axial wounds are made, the axial and circumferential methods of wounding produce quite different results. If the axial wound is made axially there is little if any reorientation by 24 h after wounding (Fig. 3a). Any migration that has taken place has done so with a considerable axial component. By 72 h (Fig. 3b) the cells at the wound edge have maintained an axial or polygonal orientation and there has been only limited circumferential migration into the wound. Once again, any migration past the wound edge appears to have a strong axial orientation, suggesting that it is axial spill-over from the irregular wound edge. The resurfacing by the migrating cells has been inefficient and gaps remain exposing bare internal elastic lamina (IEL) in the re-established area. The failure of the endothelial cells to migrate into the wound does not result from some generalized damage of the IEL because, as shown (Fig. 3b), there is extensive axial growth from an accessory artery around which the surface was denuded during the wounding procedure. Although axial regeneration from this artery has been dramatic, as

Fig. 2 Scanning electron micrographs of explant surfaces wounded as in Fig. 1a, b. The explants were stained with silver nitrate and fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) for at least 4 h. This was followed by a post-fixation in 2% aqueous OsO4 for 1 h. Fixed material was then rinsed several times in PBS, dehydrated in ethanol and critical point dried in CO2 from amyl acetate. Preparations were sputtercoated with gold and viewed with an Hitachi S-450 scanning electron microscope. Black arrows indicate the approximate edge of the wound, estimated by the location of notches cut in the explant (see c). Rubber policeman wounds were begun along a line between these notches. Open arrows mark the position of the migrating wound front at the time of fixation (approximate, for irregular fronts). a, Circumferential wound edge made by wounding axially, explant fixed 24 h after wounding (×133). b, Wound edge as in a, explant fixed 48 h after wounding (×90). c, Wound edge as in a, explant fixed 72 h after wounding. Actual notch is visible in the upper left ($\times 34$), d. Circumferential wound edge made wounding circumferentially, explant fixed 48 h after wounding (×90). In all cases, the vertical axis of the figures parallels the original blood flow axis for the vessels. The circumferential wound



edge made by circumferential wounding was produced on explants from two separate rats. All the rest have been done on explants from at least three separate rats.

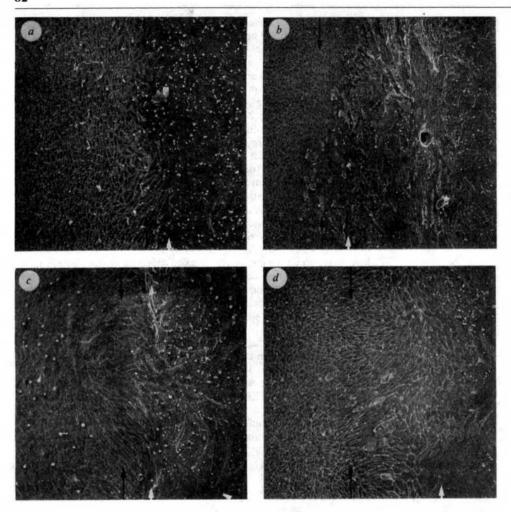


Fig. 3 Scanning electron micrographs of explant surfaces wounded as in Fig. 1c, d. The explants were preared for scanning electron microscopy in the same way as for Fig. 2, and the black arrows once again indicate the placement of the original wound edge, while the open arrows give an estimate of the extent of the endothelial migration before fixation. a, Axial wound edge made by wounding axially, explant fixed 24 h after wounding (×98). b, Wound edge as in a, explant fixed 72 h after wounding (×52). c, Axial wound edge made by wounding circumferentially, explant fixed 24 h after wounding (×82). d, Wound edge as in c, explant fixed 48 h after wounding (×55). Again, in all cases, the vertical axis of the figures parallels the original blood flow axis for the vessels.

typically seen in *in vivo* wounds, circumferential migration from the same artery has not occurred.

For an axial wound made circumferentially, endothelial cells initially elongate and migrate circumferentially (Fig. 3c). By 48 h (Fig. 3d), however, two types of orientation are visible. Endothelial cells in the area of the original wound edge are oriented circumferentially, but at the edge of the regenerating endothelial sheet the cells are oriented axially. Between 48 and 72 h no further circumferential migration takes place, and consequently, part of the original wound area remains denuded. In contrast to the results for circumferential wound edges, we have observed that the extent of circumferential orientation in these wounds is influenced by the intensity of the wound. Light wounds produce much less circumferential orientation than do heavy ones.

It seems likely that the information affecting the orientation and migration of the endothelial cells on our explants must come from either the cells next to the migrating cells or some structure or cells below the endothelium. The former theory is supported by the results from circumferential wound edges, which demonstrate only orientation parallel to the orientation of the adjacent endothelium. In contrast, on explants with axial wound edges made circumferentially, the endothelial cells are clearly able to orient, and then reorient, in each case perpendicular to the orientation of the cells which would be presumed to be the source of orientation information. We feel, therefore, that the observations are more consistent with the source being beneath the endothelial cells, and present the following working hypothesis for the involvement of subendothelial information in the orientation and migration of regenerating endothelial cells on aortic explants.

When part of the endothelium is removed, cells along the wound edge elongate before migrating perpendicular to that edge. For circumferential wound edges made axially, axial information is left intact, the elongation is pronounced and migration proceeds in a similar manner to that described for *in vivo* wound recovery². This seems to be a result of a combination of the tendency of migratory cells to invade a newly bared area (demonstrated for monolayer cultures without underlying matrix⁹) and contact guidance from axial cues⁴. In the case of circumferential wound edges made circumferentially, cells migrate in a near-normal manner but the more random orientation of cells at the migrating front suggests the partial loss of axial cues.

In the cases of wound edges which parallel the axial information, our observations support the idea that axial information not only directs endothelial migration, but may actually restrict it. For axial wound edges made axially, we believe that it is the persistent axial information which is sufficient to inhibit the elongation and migration perpendicular to the wound edge. In contrast, for axial wound edges made circumferentially, we propose that partial disruption of the axial information allows elongation and migration in a manner which seems to be due to a summation of factors similar to that for a circumferential wound made circumferentially, that is, compromised matrix information, and an heightened effect of the intrinsic tendency for cells to migrate into a bare area. Between 24 and 48 h in culture, the migrating cells of the circumferentially made axial wound edge reorient, once again aligning with the original axis of blood flow. This may indicate a regeneration of subendothelial information, or simply a change in the ability of the migrating endothelial cells to read the remaining axial information.

Other investigators have already demonstrated changes of endothelial orientation *in vivo* which must in some way involve blood flow forces^{10,11}. Our use of cultured explants has made it possible to eliminate blood flow from the factors responsible for the changes described here. However, two results from the present study seem to be important with regard to the relation-

ship between blood flow and subendothelial cues in the ultimate regulation of the orientation of endothelial regeneration in vivo. The first of these is the relative velocity of the reorientation with respect to these two variables. Changes induced by blood flow manipulation seem to require at least 3 days in vivo 10.11 and 48 h for monolayers of endothelial cells¹². Reorientation in our experiments occurs in as few as 24 h and two such reorientations can take place in 48 h. Second, our observation of the inability of the cells to maintain a migration in the circumferential direction, here in the absence of blood flow, supports data from in vivo experiments showing that endothelial cells migrate effectively against blood flow but not circumferentially3. These two points strongly suggest that there are some aspects of endothelial regeneration which are not produced by the shear forces of blood flow; that is, the structure of subendothelial information may be modified by blood flow (see ref. 4), but it is the underlying matrix which carries the imperative of orientation for migrating endothelial cells.

I acknowledge the guidance and support of Dr Judd Sheridan, valuable discussions with Dr Dale Pederson, and thank Drs David Hamilton and Michael Atkinson for advice on the developing manuscript. I thank Susan Anderson for technical training in scanning electron microscopy. This work was supported by HL 21166 to Dr J. D. Sheridan. During initial development of the techniques, R.W.J. was an NIH trainee (GM 07094).

Received 27 September 1981; accepted 12 January 1982.

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Are there purinergic receptors on parotid acinar cells?

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ATP and other purine nucleotides and nucleosides have potent regulatory (neurotransmitter-like) actions which have been attributed to interaction with a specific plasma membrane receptor¹. To date the receptor mechanisms underlying purinergic activation have been poorly characterized. One problem has been the variability of the evoked effects in different tissues. Burnstock^{2,3} proposed that much of the variability could be explained if the effects were mediated by two separate receptors, termed P₁ and P₂, with different specificities for agonists and antagonists. Receptor mechanisms have been extensively investigated in the parotid gland⁴. I now report that in that gland, ATP evokes a marked increase in membrane conductance, radioactive Rb efflux and amylase secretion. The effects of ATP are similar to those evoked by acetylcholine (ACh) and α -adrenergic agonists but are still present when cholinergic and adrenergic blocking agents are used. The latency and reversal potential of the ATP-evoked effects are comparable with those of the autonomic agonists. The ATP receptor on parotid acinar cells is of the P₂ type^{2,3}, since the order of potency of the nucleotide series was ATP>ADP»AMP, adenosine had no effect, and the response could be blocked by quinidine but not by theophylline.

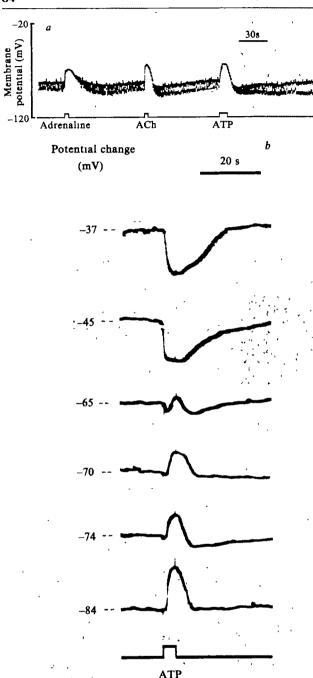
Experiments were carried out on 249 isolated parotid glands from 152 adult male mice. All glands were superfused with oxygenated physiological saline solutions at 37 °C. The acinar cell response to the various agonists was investigated using three experimental techniques: (1) single glass microelectrodes were used to record transmembrane potential and input resistance from surface acinar cells⁵; (2) the rate of Rb efflux was monitored by measuring the efflux of 86Rb from segments of prelabelled glands^{6,7}; and (3) amylase secretion was measured continuously by an on-line fluorometric method⁸. The agonists ATP, ADP, AMP, adenosine, ACh and adrenaline (Sigma) were applied by inclusion in the superfusion media, which had a final pH of 7.4. ATP, ACh and adrenaline were also applied ionophoretically from an extracellular micropipette.

The mean resting potential of the acinar cells was $-62.2 \pm$ 0.62 mV (±s.e.m., n = 74) and the mean input resistance $4.6 \pm$ $0.39 \text{ M}\Omega$ (n = 19), in close agreement with previously published values 10,11. Electrophysiological responses to ionophoretically applied ATP were observed in 69 cells from 18 glands. When the effects on the membrane of ATP and the autonomic agonists ACh and adrenaline were compared in 47 of these cells, they were all found to evoke a marked decrease in input resistance associated with an initial depolarization. This initial potential change was followed by a delayed hyperpolarization during which the input resistance returned to prestimulus levels (Fig. 1a). Such biphasic membrane responses have been previously reported in this tissue following cholinergic α -adrenergic and nervous stimulation^{9,10}

In the remaining experiments the effects of ATP were investigated in the presence of combined autonomic blockade by atropine (10⁻⁵ M), phentolamine (10⁻⁵ M) and propranolol $(5 \times 10^{-6} \,\mathrm{M})$. The autonomic blockers had no effect on the resting membrane potential and input resistance, which were $-66.3 \pm 0.67 \text{ mV}$ (n = 94) and $5.06 \pm 0.31 \text{ M}\Omega$ (n = 43) respectively. It was necessary to demonstrate the persistence of the ATP effects in the presence of autonomic blockade because the peptide hormone substance P has been shown to evoke indirect effects in this tissue by release of endogenous neurotransmitter (ACh). 11 The initial potential change of the ATP response shows the same dependence on transmembrane potential gradient as has been reported for the cholinergic and α -adrenergic responses in this tissue. Figure 1b shows the ATP effects evoked in a single cell at different levels of membrane potential. In Fig. 1c the amplitude of the ATP effects is plotted as a function of the membrane potential. The reversal potential of the ATP effect was measured in this manner on five separate occasions, having a mean of $-59.6 \pm 4.1 \,\text{mV}$ (s.d.). This reversal potential is not significantly different from the values of -58.3 ± 9.0 and -61.9 ± 8.4 mV (±s.d.) previously reported for ACh and adrenaline in mouse parotid gland 10. The latency of the effects of ionophoretically applied ATP, in the presence and absence of the autonomic blockers, was $471 \pm 33 \text{ ms}$ ($\pm \text{s.e.m.}$, n = 9) and 489 ± 58 ms (\pm s.e.m., n = 15) respectively, which is comparable with values reported previously for ACh and adrenaline in this tissue 10. The identical latency in the presence and absence of the autonomic blockers excludes the possibility of any early indirect effects11,12

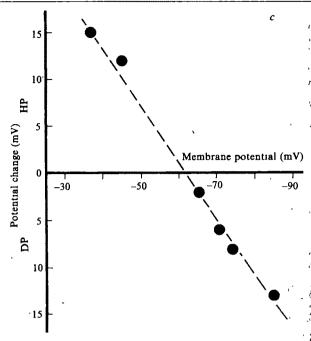
Superfusion of ATP in 55 cells from 37 glands in the presence of the combined autonomic blockers evoked a marked decrease in input resistance associated with a monophasic hyperpolarization similar to that reported following superfusion of cholinergic and α -adrenergic agonists¹³. Figure 2 shows the dose-response curve for the ATP-evoked hyperpolarizations at effective ATP concentrations of 10^{-5} – 10^{-3} M. The true dose– response curve is thought to lie to the left of the experimental one because (1) all investigations of the effects of exogenously applied ATP are complicated by hydrolysis or tissue uptake of the nucleotide²; (2) it has been reported that the α -adrenergic blocker phentolamine, at the concentration used routinely in this study to eliminate the possibility of indirect effects, will interact with the purinergic receptor¹⁴; and (3) ATP-evoked secretion in mast cells produces a similar dose-response

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curve^{15,16}, although in this case the true ATP agonist is the free form, the tetrabasic acid (ATP⁴⁻), which constitutes only a small fraction of the total ATP in solution ([ATP]). The [ATP⁴⁻] in the solutions used to obtain the curve in Fig. 2 (Ca 2.5 mM; Mg 1.37 mM) can be estimated as 2×10^{-7} – 2×10^{-5} M respectively¹⁵.

In parotid gland, as in other tissues, 86 Rb has been used as an indicator of potassium movement $^{6.7}$, and it is known that cholinergic and α -adrenergic agonists evoke a marked efflux of K (Rb) from acinar cells. Figure 3a demonstrates that when ATP is included in the superfusion media, even in the presence of the autonomic blockers, it mimics the effects of the autonomic agonists in its ability to evoke Rb efflux from the parotid tissue. Burnstock $^{2.3}$ describes two types of receptor—the P_1 and P_2 purinergic receptors—which mediate the effects of exogenous and endogenous nucleotides and which are identified by their different specificities for the nucleotide/nucleoside series and their susceptibility to blockade by different agonists. The nucleotide series in evoking Rb efflux from this tissue is ATP > ADP > AMP (Fig. 3a). Adenosine had no effect even at 10^{-2} M (12 glands, 3 experiments), and failed to evoke any electro-



ATP-evoked changes in membrane potential and conductance Recording of membrane potential and input resistance from acinar cell of mouse parotid gland. The potential changes superimposed on the record of resting membrane potential are due to repetitive injection of hyperpolarizing current pulses through the recording electrode (2 nA, 100 ms) The amplitude of these electrotonic potentials corresponds to the input resistance of the acinus Responses are seen to the ionophoretic application of the agonists adrenaline, ACh and ATP The initial potential change is due to an increase in passive membrane permeability primarily to K and Na. The resulting efflux of K+ and influx of Na+ stimulate an electrogenic Na/K pump, which gives rise to the delayed hyperpolarization seen when the input resistance has returned to prestimulus levels (for review, see ref. 20). The ATP micropipette contained 0 5-1 0 M ATP in distilled water. Ejection current was 300 nA applied for 8 s Responses were recorded with hyperpolarizing ejection charges of ≥50 nC. Depolarizing ejection charges were meffective as was ionophorers of a control solution of distilled water titrated to pH 3.0. Adrenaline and ACh ionophores were achieved by depolarizing ejection currents of 300 and 200 nA respectively for 4 and 3 s (ref. 9). Note the similar nature of the membrane response to the three agonists. b, Traces showing the nature of the ATP-evoked potential change at different levels of membrane potential in the same cell. The membrane potential was varied by application of direct current through the recording electrode. The superfusion media contained all three autonomic blockers throughout, atropine 10^{-5} M, phentolamine 10^{-5} M and propanolol 5×10^{-6} M. The dependence of the ATP-evoked effects on transmembrane potential is demonstrated by the reversal of the response from a depolarization (DP) at the higher levels of membrane potential to a hyperpolarization (HP) at the lower levels of membrane potential. c, The amplitude of the response (data from b) plotted as a function of the membrane potential. The reversal potential, the intersect with the abscissa, is about -62 mV Five such direct measurements of reversal potential were made with a mean of $-59.6 \pm 8.4 \text{ mV}$ ($\pm s.d.$).

physiological effects in any of nine cells from four glands when included in the superfusion media at 10^{-2} M. Histamine $(10^{-4}$ M) (8 glands, 2 experiments) also failed to evoke any increase in Rb efflux, eliminating the possibility that the ATP effects were secondary to release of endogenous histamine.

effects were secondary to release of endogenous histamine. Figure 3b shows that when 10^{-3} M quinidine was included in the superfusion media, ATP failed to evoke any significant increase in Rb efflux (36 glands, 9 experiments); subsequent application of ACh $(10^{-6} \, \text{M})$, still in the presence of quinidine, evokes the characteristic increase in Rb efflux (20 glands, 5 experiments). In the presence of the ophylline $(10^{-3} \text{ M}; \text{ Fig. } 3b)$ the ATP effect persists, unaffected (16 glands, 4 experiments). The same specificity was seen in electrophysiological experiments. ATP failed to evoke any effect in three cells (two glands) in the presence of 10^{-3} M quinidine. However, the purinergic effects were readily evoked in the presence of theophylline (10⁻³ M), by both superfusion (six cells, three glands) and ionophoresis (two cells, one gland) of the agonist. Quinidine blockade, especially at the concentration used, is not specific and more prolonged exposure to quinidine (35 min) abolished the effects of α -adrenergic stimulation in this tissue, as in others¹⁷.

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The persistence of the ACh effect at this time (Fig. 3b) and later would indicate that the non-specific interaction occurs at the receptor level and is not due to a generalized blockade of the effector mechanism.

In addition to the stimulus-permeability effects, cholinergic and α -adrenergic receptor activation are associated with amylase secretion, which is characterized by its dependence on extracellular calcium¹⁸. β -adrenergic stimulation, which is not associated with any of the conductance changes described above¹⁰, is a potent stimulator of enzyme secretion, but by a mechanism independent of extracellular calcium and attributed to interaction with the adenulate cyclase-cyclic AMP system¹⁹. ATP evokes a marked increase in amylase secretion (28 glands, 7 experiments) which exhibits the same dependence on extracellular calcium (20 glands, 5 experiments) as the other stimulus-permeability-coupled receptors (Fig. 4). Adenosine $(10^{-2} \,\mathrm{M})$ had no effect on amylase secretion (8 glands, 2 experiments). The effects of the antagonists on amylase secretion could not be tested as quinidine itself interacted with the fluorometric assay.

Whereas a peptide (substance P) receptor was previously reported in rat acinar cells, but appeared absent from mouse acinar cells, in this study ATP effects were readily observed in the mouse parotid gland but could not be evoked in the rat. Superfusion of ATP (10⁻³ M) failed to evoke electrophysiological effects (6 cells, 2 glands), Rb efflux (4 glands, 2 experiments) or amylase secretion (4 glands, 2 experiments).

This study of mouse parotid gland reveals for the first time that ATP can mimic the effects of cholinergic or α -adrenergic stimulation in terms of increased membrane conductance, 86Rb efflux and calcium-dependent enzyme secretion. The persistence of the ATP-evoked effects in the presence of a combination of autonomic blockers and their short latency (equivalent to autonomic activation) suggest that they are mediated by interaction of the nucleotide with a specific surface membrane receptor. The order of potency of the nucleotide series (ATP> ADP » AMP) and the suceptibility to blockade by quinidine but not theophylline indicate that a purinergic receptor of the P₂ type is involved^{2,3}. This type of purinoreceptor predominates in the gastrointestinal system². The identical nature of the membrane responses and the common reversal potential shared by the purinergic, cholinergic and α -adrenergic agonists suggest that all three effects, although mediated by separate receptors, are the consequence of the activation of the same effector

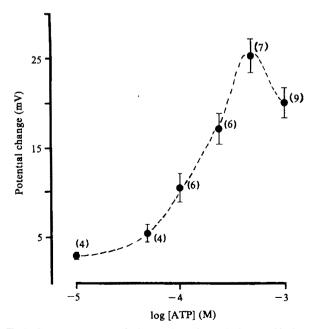
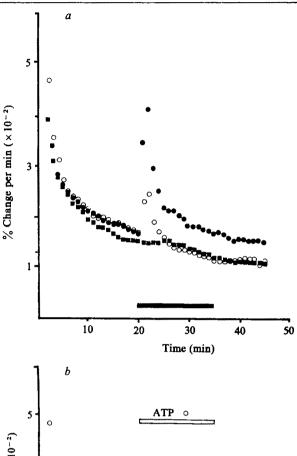


Fig. 2 Dose-response curve for hyperpolarization evoked in parotid acinar cells by superfusion with ATP $(10^{-3}-10^{-3} \text{ M})$ All experiments were carried out in the presence of the combined autonomic blockers. Figures in parentheses are the number of individual determinations. Plot is of means $\pm s.e.$



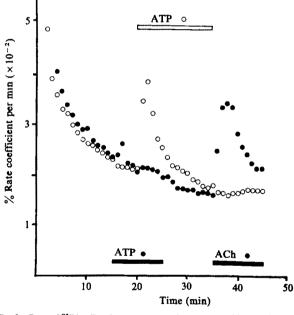
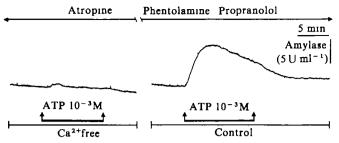


Fig. 3 Rate of ²⁶Rb efflux from segments of mouse parotid gland. In each experiment, segments of four glands were loaded with radioactive Rb for 30 mm in 1 ml of physiological salt solution containing 3-5 μCi ⁸⁶Rb. After loading the segments were transferred to a flow chamber (volume 1 ml; flow rate 2 ml min-1). The effluent was collected at 1 min intervals and subsequently analysed in a scintillation counter for ⁵⁶Rb content. The radioactivity remaining in the tissue at the end of the experiment was determined and the concentrations in the minute samples converted to rate coefficient⁶ by computer. a, Effects of purine nucleotides on the rate coefficient of ²⁶Rb efflux (given as % change per min). The agonists were applied by inclusion in the superfusion media at 20-35 min, as indicated by the bar in the figure. The autonomic blockers were present throughout the experiments. Each point is the mean of a series of experiments \bullet , ATP (10^{-3} M), n=5; O, ADP (10^{-3} M), n=3; \blacksquare , AMP (10^{-2} M), n=3. (s.e. during application of the agonists was 5-15%) The order of potency of the nucleotide series was ATP>ADP > AMP. Neither adenosine (10-2 histamine (10-4 M) had any effect. b, Effect of ATP (10-3 M) on 56 Rb efflux in the presence of quinidine (\bullet ; 10^{-3} M; n=5) and theophylline (O; 10^{-3} M; n=4). Quantitine blocks the effect of ATP applied at 15-25 min. ACh (10^{-6} M) applied in the same experiments at 35 min still evokes the characteristic increase in 86 Rb efflux. When applied at 20-35 min in the presence of theophylline, ATP evokes the characteristic increase m ⁸⁶Rb efflux. The affinities demonstrated for the agonists (ATP>ADP>AMP) and antagonists indicate that the effects are mediated by a P₂ purinergic receptor^{2,5}.



Effects of ATP on amylase release from segments of mouse parotid gland. The amylase content of the effluent from the tissue chamber (volume 1 ml; flow rate 1 ml min⁻¹) was continually assayed by an automated on-line fluorometric method⁸. The figure which is two sections of a continuous trace, shows the effects of ATP in the presence and absence of extracellular calcium from the superfusing solution. The calcium-free solution contained 10⁻⁴ M EGTA. The autonomic blockers were present throughout the experiment The ATP-evoked increase in amylase secretion is seen to depend on extracellular calcium.

mechanism. These stimulus-permeability-coupled receptors give rise to an increase in membrane permeability primarily to K and Na by a mechanism which probably utilizes Ca as a common intracellular messenger²⁰.

This work was supported by a grant from MRC to O. H. Petersen. D.V.G. gratefully acknowledges the assistance of A. R. Chipperfield with Rb-efflux experiments.

Received 7 September, accepted 18 December 1981

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Neural mechanisms for serial order in a stereotyped behaviour sequence

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A single, transient sensory stimulus can evoke a complex behavioural response in which several behavioural units occur in a serially ordered manner. For stereotyped behavioural sequences, two alternative neural mechanisms have been proposed to account for the serial ordering of behavioural units: chain reflexes1, which depend on peripheral feedback, and central pattern generators2, which attribute the sequence to interactions among central neural elements. Although long thought to be mutually exclusive, both alternatives were recently shown to contribute to simple crayfish escape responses³⁻⁵. We now demonstrate that the integration of these simple, serially ordered escape responses into the compound escape sequence of the crayfish is mediated by a third mechanism for serial order, namely the parallel activation by the same sensory stimulus of two separate behavioural units with different intrinsic reaction times.

Crayfish can escape from predators by swimming backward using a series of rapid abdominal flexions and extensions (tailflips). When escape is initiated at short latency by a sudden tactile stimulus to the abdomen, an initial stereotyped tailflip is mediated by a pair of giant axons, while all subsequent tailflips (swimming proper) are produced by a non-giant central pattern . The giant axons have been termed 'command neurones'6 because, when stimulated directly, they produce an entire, coordinated tailflip. However, we recently showed that only the flexion phase of the first tailflip is produced by central action of the giants; abdominal re-extension is a chain reflex that is triggered by sensory feedback from the flexion⁵.

Proper behavioural integration requires that the non-giant central pattern generator for swimming be turned on at the right time following the first giant-mediated tailflip. How is this achieved? We considered three possibilities. First, the giant axons might trigger the central pattern generator (CPG) via a delayed, central pathway. Second, the CPG, like the re-extension reflex, might be triggered by feedback from the first tailflip. Third, the tactile, initiating stimulus might trigger both the giant axon system and the CPG in parallel. Evidence for the third alternative was obtained by stimulating directly and selectively the giant axons of unrestrained crayfish via implanted electrodes^{4,5} and comparing the resulting escape responses with those initiated by natural stimuli.

Twenty crayfish, Procambarus clarkii (of both sexes; 3-7 cm long), were kept individually in well-aerated, 40-1 aquariums. Each animal was made to respond only once per 10 min. Approximately half of the responses were produced by lightly tapping the abdomen; the other half were produced by a direct shock to the giant axons. Responses were monitored visually and via chronically implanted recording electrodes.

The result was an almost complete dichotomy in the incidence of multiple tailflips produced by natural and electrical stimuli. Following taps, ~82% of the escape responses consisted of an initial, giant-mediated tailflip followed by one or more nongiant (CPG-mediated) tailflips. But when the giant axons were stimulated directly, only a single tailflip ensued; CPG-mediated swimming was almost completely absent, occurring in <1% of all trials (Table 1). These results mean that activity in the giant axons is insufficient to trigger the CPG either via a central pathway or via feedback. Instead, excitation arising from the tactile stimulus is necessary for CPG activation.

Is the tactile stimulus sufficient to trigger the CPG, or must it be coupled with sub-threshold excitation produced by the giant axons? In the preceding experiment, only 4% of the responses were mediated by the CPG alone, which may mean that the tactile stimulus is rarely sufficient to activate the CPG, or that the stimuli used were typically supra-theshold for both the giant axons and the CPG.

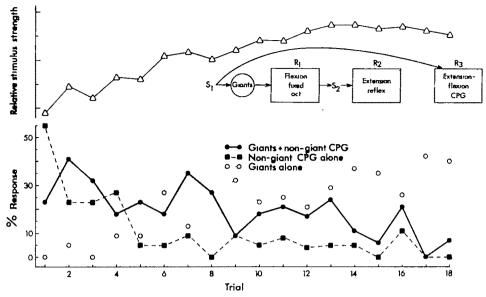
We attempted to activate the systems separately by reducing the stimulus strength so that it was just at escape threshold (50% responding), and by testing at 1-min intervals so that thresholds rose during a testing session (presumably because of habituation). Four animals were tested in this way. The results (Fig. 1) show that the tactile stimulus is frequently sufficient to trigger the CPG. Indeed, in rested animals the CPG threshold is lower than the giant axon's threshold. In the first trial, 71% of the escape responses were mediated exclusively by the CPG and 100% included the CPG (Fig. 1). (Repetitive stimulation caused the threshold for the CPG to rise relative to the LG threshold,

Table 1 Different response modes produced by central and peripheral stimulation

	Type of response Giants				
Type of stimulus	Giants	+	Non-giants		
	alone	non-giant	alone		
Shock to giant axons $(n = 445)$	99.3%	0.7%	4.0%		
Touch to abdomen $(n = 453)$	13.9%	82.1%			

Fig. 1 The block diagram illustrates the relationships between the units of behaviour that make up the crayfish escape response. The initiating stimulus activates R1 and R3 in parallel, but R3 occurs only after a delay. R2 is a chain reflex that depends on sensory feedback from R₁. The graph shows differences in initial thresholds and habituation rates of giant-mediated responses $(R_1 + R_2)$ and non-giant(CPG)mediated responses (R3). In the experiment shown, each of four animals was tested at 1-min intervals on five successive days. Thus each trial represents the average obtained from ~20 stimuli, given as per cent The responsiveness. stimulus strength (top) was increased over trials in order to maintain a total escape response rate close to 50%. Total response rates varied between 77% (trial 1) and 45% (trial 12). By

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keeping the stimulus close to threshold we were able to demonstrate the separate response pathways Stimulus strength is indicated in relative units (peak initial amplitude of the output of a mechano-electrical transducer which was coupled to the mechanical stimulator). The three main results are: (1) the systems can be evoked independently; (2) the CPG threshold is initially the lowest; and (3) the CPG habituates more rapidly.

thus solo activity of the CPG quickly waned, with solo activity of the giant system eventually predominating.)

Given that the same stimulus can activate both behaviours in parallel, how is the serial order of the compound behaviour achieved? Previous results can now be re-interpreted to show that an intrinsic difference in response latency is an important mechanism for the sequential ordering of the behavioural units. The giant axon pathway has obviously evolved to produce responses of very short latency: the pathway is short, its neurones are large, and all known synapses except those of receptors and motoneurones appear to be electrical^{7,8}, resulting in a modal response latency of <7 ms. In contrast, the minimum latency for activation of the non-giant CPG is ~50 ms and the modal latency is >150 ms (see ref. 4). (The neural basis for the long latency of the CPG has not yet been investigated.) The latency values reported by Wine and Krasne⁴ have been confirmed in our experiments, which are based on a much larger sample size. For 180 giant-mediated tailflips, the mean latency was 6.3 ± 0.2 ms. For 63 exclusively non-giant responses, the mean latency was 240 ± 21 ms. The mean duration of the giantmediated tailflip (flexion and re-extension), occurring in isolation, was 190 ± 21 ms. Thus, it is clear that the typical latency to the beginning of CPG-mediated tailflips is long enough to allow the first two behavioural units to occur in the interim.

These results extend our understanding of how serial order is achieved in stereotyped behaviour. A previous hypothesis¹ to explain how a transient sensory stimulus could lead to ordered behavioural responses proposed that sequencing occurred because each reflex caused feedback appropriate to trigger the next reflex. Convincing examples of chain reflexes are rare^{5,9} and so far have been restricted to episodic behaviours. A second hypothesis is that order is imposed by a central pattern generator, envisaged as one or more neural oscillators. This hypothesis has been confirmed for every rhythmic behaviour whose production has been analysed carefully². Both these mechanisms are used in simple crayfish escape responses. In addition, a third mechanism—parallel activation by the same sensory stimulus of two separate neural subsystems with different reaction times—is responsible for the integration of a chain reflex and a central pattern generator into a seemingly unitary complex behavioural act.

What is the adaptive significance of the parallel processing strategy? We know that the giant axon system ignores the laterality of the stimulus and produces a bilaterally symmetrical response, whereas non-giant responses are laterally asymmetric and can steer the animal away from threat^{4,10}. Therefore, the parallel strategy may be required because the stimulus contains information that is used by the second system but not by the first.

It seems likely that reaction-time mechanisms for serial order occur widely. For example, the timing of two distinct components of the hours-long, hormonally-induced eclosion behaviour of a moth appears to rely on a reaction-time mechanism¹¹. Thus this general type of mechanism seems capable of ordering behavioural units into complex sequences of action on time scales which vary from milliseconds to hours.

We thank G. Hagiwara for assistance, J. Ruby for preparing the manuscript, and M. R. Plummer, F. B. Krasne and D. Glanzman for helpful comments. This work was supported by NSF grant BNS 78-14179 (J.J.W.) and by DFG grant Re 484/1 (H.R.).

Received 9 November 1981, accepted 19 January 1982

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FMRFamide neuropeptides simultaneously increase and decrease K⁺ currents in an identified neurone

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Phe-Met-Arg-Phe-NH₂, or FMRFamide, discovered in ganglia of the clam Macrocallista nimbosa1, has potent cardio-stimulatory activity in some molluscan species, but is cardio-inhibitory in others, and produces contractions of certain non-cardiac muscles². A peptide with similar properties is present in the ganglia of the snail Helix3, and is thought to differ from the Macrocallista peptide in having one or more additional amino acids combined with the terminal free amino nitrogen. An

homologous opiate peptide, Tyr-Gly-Gly-Phe-Met-Arg-Phe (or YGGFMRF, using single letter abbreviations4), was identified in adrenal medullary granules and the striatum⁵. YGGFMRFamide has chromatographic properties more similar, though not identical, to the Helix peptide than FMRFamide itself (D. A. Price, M. J. Greenberg and G.A.C., unpublished). FMRFamide has potent and complex effects on Helix neurones. One neurone in each cerebral ganglion, the C1 or giant serotonin neurone (GSN), is hyperpolarized by FMRFamide at the resting membrane level, but depolarized at less negative potentials⁶. I show here that YGGFMRFamide has similar effects to FMRFamide and examine further the ionic mechanisms underlying the two responses of the GSN. The results indicate that the hyperpolarizing, outward current response results from an increase in conductance to K+ whereas the depolarizing response, which was recorded as a reduction in net outward current, is due to the suppression of a Ca2+ activated K⁺ current.

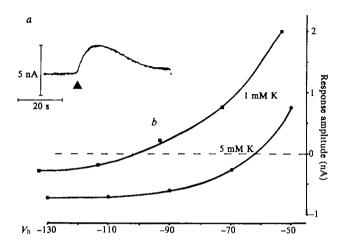
In most experiments the neurone was voltage-clamped using a Dagan 8100 single electrode system with K-acetate or KCl electrodes of $\sim 1~M\Omega$ (see ref. 7). However, responses were also recorded with a two-electrode system which incorporated either the amplifier of a Tektronics 502A oscilloscope or an RS-7611 C-Moss amplifier, in the feedback circuit. Physiological solution (NaCl 80 mM, KCl 5 mM, CaCl₂ 7 mM, MgCl₂ 5 mM, Tris buffer 5 mM, final pH 7.8) was allowed to flow continuously over the isolated cerebral ganglia preparation pinned to the base of a 0.7-ml chamber. The peptides were applied locally to the GSN either by iontophoresis (4 mM in distilled water) or by pressure ejection from a pipette (50–100 μ M concentrations of each peptide).

Each peptide hyperpolarized the GSN at the resting potential. The size of the hyperpolarizing response was increased when the external K⁺ concentration was reduced, but remained unchanged when the external Cl⁻ concentration was reduced. Under voltage-clamp, the reversal potential of the peptide-induced outward current was increased by -35 to -38 mV when the external K⁺ was changed from 5 to 1 mM (see, for example, Fig. 1b). This shift in reversal potential is close to the theoretical value of -41 mV and indicates that K⁺ is primarily, if not exclusively, involved in the hyperpolarizing response to each peptide, although the reversal potential of the response seems to be more positive for this neurone than that recorded for K⁺

Fig. 1b). This shift in reversal potential is close to the theoretical value of -41 mV and indicates that K⁺ is primarily, if not exclusively, involved in the hyperpolarizing response to each peptide, although the reversal potential of the response seems to be more positive for this neurone than that recorded for K+ responses in some other molluscan neurones (see, for example, ref. 8). The outward current response was depressed with tetraethylammonium bromide (TEA) at 30 mM, but was not completely blocked even with 100 mM TEA. It was also reduced by cooling to 4 °C. The other action of the peptides was observed at potentials less negative than the resting level. This apparent inward current response increased in size as the membrane was depolarized. Frequently a combination of both outward and 'inward' current responses was observed (Fig. 2c, d). TEA at 30 mM greatly reduced the response due to the increase in K⁺ conductance^{9,10} and revealed an enlarged, or in some cases a previously undetected, response which was inward in sign (Fig. 2a). The size of the apparent inward current response was not reduced when the external NaCl was replaced with sucrose or glucosamine hydrochloride, suggesting that neither Na⁺ nor Cl⁻ is directly involved in the response. An involvement of Ca²⁺ did, however, seem likely, as CoCl₂ at \geq 1.5 mM reversibly abolished this response (see Fig. 2b and ref. 11). On the other hand, when BaCl₂ was substituted for most of the CaCl₂ (6 mM BaCl₂ 1 mM CaCl₂), the response was also abolished. This suggested that the response was not simply due to an influx of Ca2+, but that it could result from a suppression of a K⁺ current, as Ba²⁺ can carry the Ca²⁺ current in snail neurones¹² but blocks K⁺ currents¹³. A role of K⁺ was also suggested by the results of 2 out of more than 60 experiments made with FMRFamide, in which only the inward current response was recorded. In these two experiments, the size of the response at a holding potential of -30 mV was greatly increased

on reducing the external K⁺ concentration from 5 to 1 mM.

There is some variability in the sensitivity of the delayed K⁺ and the Ca²⁺ activated K⁺ currents to different concentrations of TEA in molluscan neurones. In some neurones, both currents are equally affected whereas in others the Ca²⁺-activated K⁺ current seems to be less susceptible^{9,10}, which could explain the preferential reduction in the outward current response with



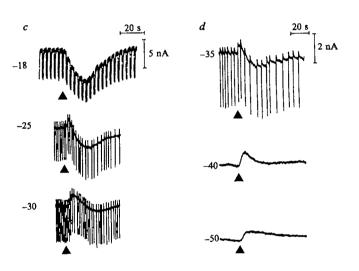
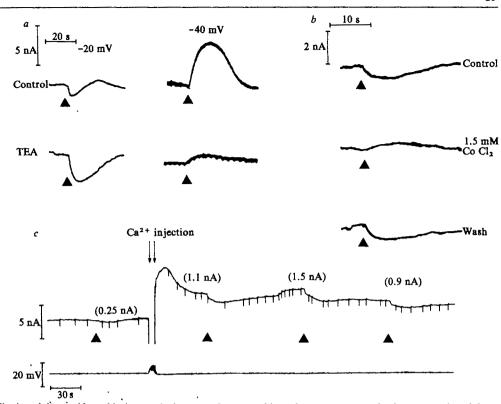


Fig. 1 a, An example of the outward current response recorded when FMRFamide was locally applied to the GSN at a holding potential of -50 mV. b, Influence of holding potential and extracellular K⁺ concentration on the size and sign of this response observed in another experiment. The reversal potential in normal medium (5 mM K) was ~-62 mV. When the extracellular K⁺ concentration was reduced to 1 mM, the reversal potential increased to ~-100 mV The nonlinear relationship between response and membrane potential is similar to that previously shown for a carbachol-evoked K+ response in Aplysia by Ginsborg and Kado⁸. c, FMRFamide responses recorded at depolarized holding potentials. At -30 mV, the outward current response was shortened to give a small response, inward in sign. At -25 mV, the 'inward current' response was greatly increased and the outward current response was reduced. At -18 mV, the response seems to consist of an 'inward current' response alone. During this recording, hyperpolarizing command voltage steps were repeatedly applied across the membrane and the resulting changes in current recorded During the action of the peptide, there appears to be a reduction in current passage These recordings should be compared with the recording in a which was obtained from the same neurone (The downward deflection on the current records at -25 and -30 mV holding potentials are unclamped spikes originating in the axon, at some distance from the site of recording.) d, Similar responses of another neurone to locally applied YGGFMRFamide. At -50 mV, there seems to be only an outward current response At -35 mV, the response was predominantly inward in sign. The outward current response was shortened by the onset of the inward current reponse in the recording made at the holding potential of $-40\,\text{mV}$. Note that in the traces shown in c and d, and also in Fig. 2, responses are recorded on top of steady outward currents. Such background currents varied to some extent from cell to cell, but with a resting potential of -50 mV they were of the order of 1.5 nA at -40 mV, 5 nA at -30 mV and 20 nA at -20 mV

Fig. 2 a, Influence of 30 mM TEA on the responses of the GSN to FMRFamide applied at holding potentials of -20 and -40 mV. At -20 mV, the control response was a composite of currents which were inward outward in sign, whereas at -40 mV the response seems to be purely outward. Exposure to TEA for 8 min, reduced the outward response at -40 mV and unmasked a response which was inward in sign at -20 mV. b, Influence of 1.5 mM CoCl₂ solution on the response. At the holding potential of -20 mV, the response was inward in sign. Exposure to the CoCl₂ solution reduced the background outward current observed at -20 mV by about 10 nA. It also abolished the inward current response and unmasked a small outward current response The effect of the CoCl2 was reversed by washing, c. Effect of locally applied YGGFMRFamide on the increased K⁺ current evoked by current evoked by intracellular injection of Ca2+. Upper trace is membrane current and the lower trace membrane voltage. The neurone was voltage-clamped at -45 mV and the peptide applied locally to the neurone. Before injecting Ca²⁺, there were only small inward current responses to the peptide. (The outward current response had been depressed with 30 mM TEA.) Injection of Ca²⁺ (40 nA for ~6s) resulted in an increase in the background outward current, that

[**4**.



is, the Ca2+-dependent K+ current. Application of the peptide at this time resulted in a greatly increased inward current response, that is, a suppression of the increased outward K+ current. The amplitude of each peptide response is indicated above the upper trace

30 mM TEA on the GSN (Fig. 2a). Of the two compounds, the YGGFMRFamide was the more potent in inducing the response which was inward in sign but less potent with respect to the outward current response.

These data suggested that the apparent inward current response could result from a suppression of a Ca2+-activated K+ current. Support for this view was obtained by testing the effect of the peptides on the increased K+ current evoked by intracellular injection of Ca²⁺ in the presence or absence of TEA. In these experiments, Ca²⁺ were injected iontophoretically using a WPI iontophoretic programmer. An electrode containing 0.5 M CaCl₂ was inserted in the neurone and a positive current of 40 nA passed for 5-15 s with respect to another micropipette placed in the medium. The cell was voltage-clamped during the injection and the membrane potential therefore remained unchanged, or was only shifted by about 5 mV during the injection period. The Ca²⁺-evoked outward current response was reversed in sign at about -65 mV in normal medium and was reversed at more negative potentials in medium with reduced KCl, as previously observed in other neurones14, establishing that the Ca2+-evoked current was due to K+. An example of an experiment showing the effect of the heptapep-tide amide on the increased outward current evoked by Ca²⁺ injection is seen in Fig. 2c, in which the neurone was clamped at -45 mV and a small inward current recorded before injecting Ca²⁺. Following Ca²⁺ injection, there was an increase in outward K+ current and the inward current effect of the peptide was potentiated as much as six times. As the increased outward K+ current declined, the effect of the peptide was reduced. It was also observed that the peptide reduced the inward current evoked by Ca2+ injection when the neurone was voltageclamped at -90 and -100 mV.

From these data it is concluded that both FMRFamide and YGGFMRFamide increase one K⁺ current, which has properties of the delayed K⁺ current^{9,10}, but suppress another, probably the Ca²⁺-dependent K⁺ current described by Meech^{14,15}. Interestingly, the polypeptide apamin, a neurotoxin of bee venom, has been reported to block the Ca2+-dependent

K⁺ channels in hepatocytes and intestinal smooth muscle¹⁶. Muscarinic agonists also suppress a K+ current, the M-current in frog sympathetic neurones, although this current is not Ca2+dependent¹⁷. The significance of the dual actions of FMRFamide and YGGFMRFamide is not known, but it is possible that if the two types of action occurred on different axonal processes of the GSN there could be a suppression of transmission via the routes on which increased conductance to K⁺ occur, but a potentiation of transmission on the routes which respond with a reduction in the Ca2+-dependent K+ conductance. The GSN has a large number of axonal processes, many of which run in parallel for long distances along some nerve trunks18. It is thought that FMRFamide and related peptides may act as hormones in molluscs², and immunoreactivity to FMRFamide has been detected histochemically in gastropod neurones19.

I thank Mr B. Powell for technical support, Professor B. L. Ginsborg for helpful comments and Drs D. A. Price and M. J. Greenberg for the gift of YGGFMRFamide.

Received 5 November 1981, accepted 19 January 1982.

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Selective blockage of voltage-dependent K⁺ channels by a novel scorpion toxin

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Blocking agents of high selectivity are crucial in defining both physiologically and biochemically the molecular components that control membrane excitability. To obtain such probes for voltage-dependent ion channels, we have examined the venom of several American scorpions for the presence of polypeptide neurotoxins having the required properties. We report here that using voltage-clamped giant axons of the squid Loligo vulgaris we have identified in the venom of the scorpion Centruroides noxius Hoffmann a polypeptide (fraction II-11) that specifically depresses the peak permeability of K+ channels without affecting their voltage-dependent open-close kinetics. The venom also contains a polypeptide toxin (fraction II-10) that specifically depresses Na+ peak permeability with only minor effects on the activation-inactivation kinetics. Furthermore, the physiological effects of the whole venom on the squid giant axon can be assigned quantitatively to the combined action of the two polypeptides.

The venom of *C. noxius* Hoffmann was purified as described previously¹. Using gel permeation and ion-exchange chromatography, we isolated seven major fractions containing polypeptides toxic to mice (fraction II-8 to II-14). Of these only the minor fractions II-10 and II-11 (5 and 2%, respectively, of the total venom protein) exerted significant effects on the electrophysiological properties of the squid giant axon in the concentration range tested. Fractions II-10 and II-11 each contain only one polypeptide, but it is possible that the former also contains very small amounts of a contaminant. The N-terminal sequences of toxins II-10 and II-11 were shown to be respectively Lys-Glu-Gly² and Thr-IIe-IIe (L.D.P., unpublished observation).

Figure 1a-c shows the reversible effects of toxin II-11 on the K^+ current, I_K , of a tetrodotoxin (TTX)-poisoned squid giant axon voltage-clamped as described elsewhere3,4. On step depolarization, toxin II-11 (1.4 µM) applied externally reversibly depressed the K^+ permeability, g_K , without affecting its voltage dependence (Fig. 1d). Potassium permeability was depressed in a dose-dependent manner (Fig. 1e), suggesting interaction of the toxin with specific receptor site(s) associated with the K⁺ channel. This view is further supported by the binding and dissociation kinetics (Fig. 1f) at two different toxin concentrations. The rate of toxin association increased with increasing toxin concentration whereas the time constant of dissociation remained constant⁵. In contrast to the effects on K⁺ channels, toxin II-11 had no significant effect on Na+ peak permeability when tested separately on three axons internally perfused with Cs ions to block K⁺ currents. In addition, neither Na⁺ nor K⁺ permeability were affected by intracellular application of toxin II-11 (1.4 µM).

Only one other polypeptide (fraction II-10) of the *Centruroides noxius* venom exerted a sizeable effect on ion channels of the squid giant axon. Figure 2 shows that toxin II-10 interacted with receptor site(s) at the Na⁺ channel, thereby reducing peak Na⁺ conductivity in a dose-dependent manner (Fig. 2d), independent

dently of the voltage (Fig. 2c). The activation kinetics were influenced only marginally (Fig. 2a, b) and the maintained current levels were affected only at higher concentrations (data not shown). However, both fast and slow inactivations were unmodified and neither the holding potential nor the external Na⁺ concentration changed toxin binding⁶⁻¹¹. Together with its reversible action, toxin II-10 thus seems to have advantages over other scorpion toxins as a probe for Na⁺ channels.

In experiments using whole venom, the actions of the venom were fully reversible and the same as those ascribed to toxins II-10 and II-11, and as observed previously for a different scorpion venom¹², the size but not the kinetics of gating currents was modified by the venom.

Various effects on voltage-dependent ion channels have been reported for scorpion venoms and purified toxins⁶⁻¹⁵. Na⁺ channel blockage is rather complex and varies with the scorpion

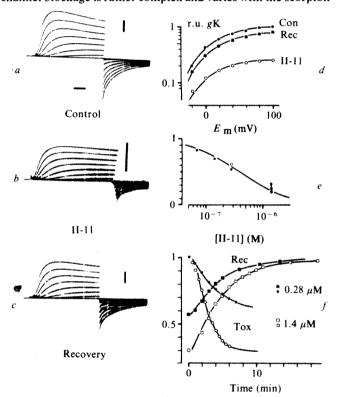


Fig. 1 Action of toxin II-11 on K^+ channels. a-c, I_K recorded at various test potentials (-20, 0, 20, 40, 60, 80 and 100 mV) before, during and after application of 1.4 µM of the toxin. Traces in b were obtained 10 min after exposure to II-11 and traces in c, 22 min after the onset of recovery. Scale bars, 1 mA and 2 ms. $E_b = -70 \text{ mV}$, $E_c = -90 \text{ mV}$ for 80 ms. Successive stimuli were separated by 3 s intervals. Positive feedback and analog compensation for leakage and capacitative currents were used. In (mM): KF (317); sucrose (307); K phosphate (45), pH 7.2. Out (mM): NaCl (435); KCl (10); CaCl₂ (10); MgCl₂ (40); Tris-HCl (20), pH 8.0; TTX (3×10⁻⁷ M). $T = 5 \pm 1$ °C. d, Instantaneous g_K in relative units (r.u.) associated with traces a, b and c as determined from the current jumps produced by step repolarizations to $E_{\rm h}$ at the end of the test pulses. The curve for control (Con) data was drawn by eye, the others were obtained by vertical shift of the same line. Rec, recovery, e, Dose-response curve of toxin II-11. Ordinate, relative $g_{\rm K}$ at $E_{\rm m}=100$ mV. The solid line is the result of a curve fit using $K_{\rm D}=390$ nM and the equation: $(1+10^{\log{\rm [TX]-\log{K_{\rm D}}}})^{-1}=g_{\rm K}({\rm TX})/g_{\rm K}({\rm C})$, where [TX] is toxin concentration, and $g_{\rm K}({\rm C})$ and $g_K(TX)$ the value of potassium conductance for the control and in the presence of toxin, respectively. KD is the equilibrium dis-

sociation constant of the reaction $TX + R \rightleftharpoons TX - R$; where R is the

concentration of receptor sites. f, Onset and offset of toxin binding measured at $E_{\rm M}=+100$ mV. Ordinate, $g_{\rm K}(t)$ normalized to the value at time zero. Solid lines are exponentials with time constants of 120 s $(1.4~\mu{\rm M})$ and 240 s $(0.28~\mu{\rm M})$ for the onset and 300 s for the offset. Tox, toxin. For clarity, complete recovery was assumed and records in c were normalized to 1 accordingly.

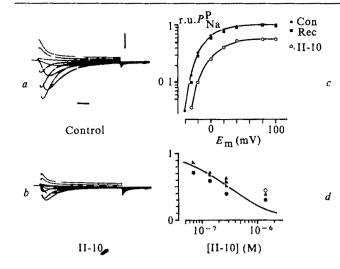


Fig. 2 Action of toxin II-10 on Na⁺ channels. a, b, Na⁺ currents recorded before and during application of 280 nM of toxin II-10. Traces in b were obtained after 14 min exposure to fraction Π -10. Recovery was complete within 35 min (not shown). Scale bars, 1 mA and 2 ms. The axon was held at $-70 \,\mathrm{mV}$ (E_b) and preconditioned to -90 mV for 80 ms (E_c) before delivering test potentials (E_t) : -40, -30, -20, 0, 20, 40, 60, 80 and 100 mV. Successive stimuli were separated by 2 s. In (mM): CsF (267); NaF (50), sucrose (307); K-phosphate (45), pH 7.2. Out (mM): same as in Fig. 1 but without TTX. T = 5 °C. c, Relative P_{Na}^{p} , peak sodium permeability, of records a and b as derived using the Goldman-Hodgkin-Katz equation 19 ; $E_{\rm Na} = 58 \, {\rm mV}$. The solid line drawn through the open circles is the same as that drawn to fit the control data but scaled in amplitude and shifted 10 mV along depolarizing voltages. d, Dose-response curve of toxin II-10. Different symbols denote different axons. Ordinate, relative P_{Na}^{P} at $E_{m} = 100 \text{ mV}$. Abscissa, molar concentration of toxin.

species^{8,15}. K⁺ channel blockage has only been described together with Na⁺ channel blockage for whole Scorpion venom^{6,13} and a toxic fraction of another venom¹⁴. Thus the important finding of the present study is that toxin II-11 acts exclusively on K⁺ channels without any significant effect on Na⁺ channel characteristics. In addition, K⁺ channel blockage is complete, independent of the applied voltage and fully reversible. These properties make toxin II-11 an excellent tool for physiological and biochemical studies of the K+ channel of excitable cells.

Compared with TTX binding to Na⁺ channels⁵ and snake toxin binding to the acetylcholine receptor^{16,17}, toxins II-11 and II-10 bind to their target sites on the squid axon with relatively low affinity ($K_D = 300 \text{ nM}$ and 390 nM, respectively) and with small association rate constants. This may be due to conformational rearrangements of the toxins required during attachment to the ion channels of the squid. More typical preys of the scorpion may have channels to which binding requires no such conformational adjustments and hence may have a larger association rate constant and lower $K_{\scriptscriptstyle \mathrm{D}}^{-18}$

Despite these minor reservations, polypeptides II-11 and II-10 seem to have advantages over previously described probes for the study of voltage-dependent Na+ and K+ channels. They have simple mechanisms of action, are fully reversible and, most important, II-11 is the first polypeptide toxin found to affect the voltage-dependent K+ channel of excitable membranes.

We thank Dr P. L. Testa for assistance in computer programming and data evaluation. L.D.P. is a fellow of the Humboldt Foundation at the Max-Planck Institut, Dortmund, FRG.

Received 26 October, accepted 15 December 1981

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Triphosphoinositide increases glycoprotein lateral mobility in erythrocyte membranes

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The finding that the turnover of the anionic phospholipid, phosphatidylinositol (PI), increases with increased synaptic activity has long stimulated interest in the possible functional roles of this phospholipid and its phosphorylated products, phosphatidylinositol 4,5-biphosphate (triphosphoinositide or TPI) and phosphatidyl-myoinositol 4-phosphate (diphosphoinositide or DPI)2. TPI metabolism is altered by a variety of cellular stimuli², but no specific function of the lipid has been identified. However, in many instances (see, for example, refs 3-6) a correlation has been observed between changes in PI metabolism and membrane structure. We have recently correlated membrane macro-viscosity with the lateral mobility of membrane glycoproteins⁷, and have shown that 2,3-diphosphoglycerate (2,3-DPG), which is similar in structure to TPI, increases glycoprotein lateral mobility. We now report that TPI also increases the lateral mobility of glycoproteins when added to erythrocyte membranes, and suggest that TPI acts similarly to other polyanions by disrupting the erythrocyte membrane skeleton.

The method of fluorescence redistribution after photobleaching (FRAP) was used to measure the lateral mobility of erythrocyte membrane glycoproteins labelled with dichlorotriazinylaminofluorescein (DTAF) as previously described8-10. We have recently shown that fluorescence photobleaching does not alter the lateral mobility of the band 3 glycoprotein in erythrocytes¹¹. The fluorescent label was primarily (65-75%) on band 3, the major anion channel of the erythrocyte, with the remainder on glycophorin and an unknown component8

Erythrocytes were lysed (1:100) in hypotonic solutions (1 part phosphate-buffered saline (PBS, 140 mM NaCl, 10 mM Na₂HPO₄, pH 7.4) and 2 parts 5 mM Na phosphate pH 7.4 with 1 mM EDTA) containing sonicated phospholipid suspensions of PI (Sigma) or ³²P-labelled DPI and TPI at different concentrations. 32P-TPI and -DPI were prepared from rat brain or from erythrocyte membranes by the method of Schacht¹², or by preparative TLC. Lipid preparations were judged to be 90% of the designated phospholipid (see Fig. 1) by two different TLC systems using commercial standards of TPI and DPI (Sigma). Sonication was performed for 10-15 min at 24 °C under N₂ in a bath sonicator to produce a clear suspension. After lysis of the cells in the presence of the lipid, membranes were incubated for 10 min at 37 °C to allow lipid incorporation and binding to occur.

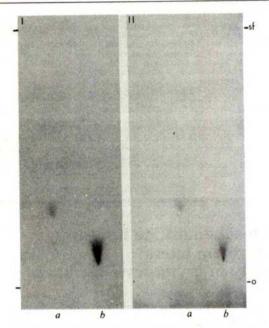


Fig. 1 TLC of 32 P-labelled DPI (a) and TPI (b) prepared by the method of Schacht¹². I is an autoradiogram and II is the material visualized by a modified Dittmer-Lester spray¹⁸. After purification, the column fractions were dried under N_2 and spotted on silica gel H (Sigma, without oxalate, 0.25 mm thick layer). Plates were developed with 1-propanol, 4 M ammonium hydroxide $(2:1)^{19}$. Sigma standards of TPI and DPI had R_F values of 0.19 and 0.33 in this system.

FRAP measurements were performed on the membranes after incubation without washing. The presence of increasing amounts of TPI in the lysis medium increased the apparent diffusion coefficient of the integral protein-bound label by more than twofold (Fig. 2). The range of diffusion coefficients for the control sample without sonicated lipid was $2.1-9.5 \times 10^{-11}$ cm² s⁻¹ whereas with 10^{-6} M TPI the range was $9.4-44 \times 10^{-11}$ cm² s⁻¹. In a paired *t*-test the average values were significantly different (P > 0.001). Figure 2 clearly shows that TPI is much more effective than DPI in increasing glycoprotein lateral mobility.

The protein diffusion coefficient is unchanged when TPI is added to erythrocytes which are swollen but not lysed in a hypotonic solution (equal parts PBS and 5 mM PO₄, pH 7.4, with 1 mM EDTA) $(4 \times 10^{-11} \, \text{cm}^2 \, \text{s}^{-1})$ compared with $4 \times 10^{-11} \, \text{cm}^2 \, \text{s}^{-1}$ for control in this experiment). This suggests that

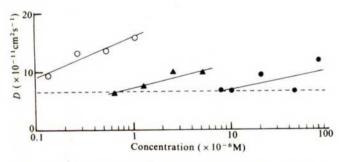


Fig. 2 The apparent lateral diffusion coefficients (D) of DTAF-labelled membranes are plotted against the concentration of TPI (\bigcirc) and DPI (\blacktriangle) , prepared by the method of Schacht¹², and PI (\blacksquare) ; Sigma). Each point is the average of four determinations. The control value (n=19) is shown by the dotted line. After pretreatment with diisopropylfluorophosphate and labelling with DTAF, cells were lysed by diluting 1:100 in sonicated suspensions of the lipids in 46 mM NaCl, 5 mM Na₂HPO₄ and 1 mM EDTA (pH7.4). Membranes were preincubated for 10 min at 37 °C before the FRAP measurement.

the active agent must reach the interior membrane surface. The observed increases in protein diffusion could not have been caused by contaminants of the lipid preparations (ammonia, ammonium acetate and neomycin). Ammonia and ammonium acetate will readily cross membranes and thus would be expected also to affect unlysed membranes. Neomycin and other amines have been shown previously to reduce the lateral mobility of membrane glycoproteins⁸. In addition, TPI prepared by preparative TLC of erythocyte membranes also caused an increase in the lateral mobility.

By analogy with previous results with 2,3-DPG, we suggest that TPI increases the lateral mobility by disrupting linkages in the erythrocyte membrane skeleton. Both TPI and 2,3-DPG have the same –5 valency, and a similar charge group structure (Fig. 3). Both are effective in increasing the lateral mobility of membrane proteins only when they have access to the cytoplasmic surface of the membrane, the surface of the extrinsic membrane matrix⁷. In many related studies, we and others have observed that 2,3-DPG and other polyanions disrupt the spectrin-actin and band 4.1 complex which comprises the erythrocyte membrane matrix¹³⁻¹⁵.

The major difference between the 2,3-DPG and TPI experiments is in the concentrations needed to produce the effects observed: 2,3-DPG will disrupt the erythrocyte membrane matrix and increase glycoprotein mobility when present in

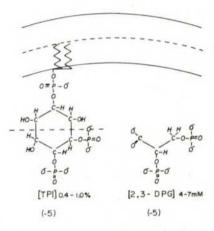


Fig. 3 The chemical structures of the lipid head group of TPI and 2,3-DPG are shown with their valency and normal in vivo concentration in erythrocytes. The concentration of TPI is expressed as a percentage of total erythrocyte lipid.

millimolar concentrations, whereas TPI is effective at the micromolar level. This difference could be explained if TPI is incorporated into the membrane bilayer, thus serving to increase its effective concentration at the membrane surface where the spectrin network is located. To assay for incorporation into cells we lysed the cells in the presence of ^{32}P -TPI. After incubation for 10 min at 37 °C, the membranes were placed on a linear sucrose gradient (1–30% sucrose in 5 mM PO₄, pH 7.4, with a 50% sucrose cushion) and centrifuged to equilibrium (30 min at 110,000g). The low ionic strength relysed the membranes, which would then float at a density of $\sim 1.15 \ \mathrm{g \, ml^{-1}}$. With both 4 and 40 μ M TPI per ml in the incubation mixture, 10% of the TPI was associated with the membrane fractions (1% cells). It is not known whether all this lipid is integrated into the membrane bilayer but it is expected that a fraction of it will be.

This information allows us to calculate the total amount of cell-associated TPI. At a concentration of 10^{-6} M, there are $\sim 10^5$ TPI molecules per swollen red cell volume, whereas in the erythrocyte membrane there are normally 10^6 TPI molecules per membrane (0.5% of total phospholipid). After incubation, approximately the equivalent of 10 cell volumes of TPI is associated with the membranes, doubling the amount of TPI in

the membrane. If some of the TPI is complexed with glycophorin^{16,17}, the added TPI may increase the amount of free TPI by more than twofold. The binding of TPI to the membrane bilayer through its hydrophobic tail will serve to increase its concentration at the membrane surface where the spectrin network is located; 1-2×10⁶ molecules of TPI corresponds to an effective concentration of 4-8 mM in a 50 Å space on the internal surface of the membrane, which in turn is the concentration range where 2,3-DPG is effective.

Thus, TPI is a potent activator of glycoprotein lateral diffusion in erythrocyte membranes, and probably acts by disrupting linkages in the erythrocyte membrane skeleton. This effect may have important consequences for such vital red cell characteristics as cell shape and deformability. In a wider context, we suggest that TPI may have a similar effect on membrane skeletal complexes in other types of cells.

This work was supported by NIH grants HL-23795 (M.P.S) and GM-28250 (D.E.K.). M.P.S. is an Established Investigator of the American Heart Association.

Received 26 October 1981; accepted 6 January 1982.

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Phosphorylation inhibits guanine nucleotide exchange on eukaryotic initiation factor 2

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There is extensive evidence that protein synthesis in reticulocyte lysates is inhibited during haem deficiency because of phosphorylation of initiation factor eIF-2 by a protein kinase (the haem-controlled repressor, HCR)¹⁻⁵. Other protein kinases, which are activated by low concentrations of double-stranded RNA, have also been identified in reticulocytes 1,6 and in extracts from interferon-treated cells⁷⁻¹⁰, and these phosphorylate an identical site on eIF-2 ¹¹⁻¹². Despite the extensive documentation of such enzymes, however, the precise mechanism of inhibition has remained unclear, mainly because phosphorylation fails to affect many partial reactions of polypeptide chain initiation 13,14. We present here evidence that the exchange of GTP for GDP bound to eIF-2 is inhibited following phosphorylation of the factor. Such an effect may inactivate eIF-2 by impairing the ability to recycle between successive rounds of protein synthesis.

It has been proposed previously that the recycling of eIF-2 between successive initiation events, rather than the formation

of initiation complexes per se, is impaired by phosphorylation. Little evidence for this, other than indirect kinetic arguments¹⁵ has, however, been presented. We have been investigating the roles of the guanine nucleotides, GTP and GDP, in regulating eIF-2 activity and have previously suggested that an important step in the recycling of the factor is the need to exchange GTP for GDP^{16,17}. The former nucleotide is required for formation of pre-initiation complexes between eIF-2, initiator Met-tRNA₁, 40S ribosomal subunits and mRNA^{13,14,18} and is then hydrolysed to GDP and phosphate on or before joining of the 60S ribosomal subunit¹⁹. The eIF-2 is released from the ribosome at this stage and may remain associated with the GDP, for which it has a high affinity²⁰. GDP is a potent inhibitor of eIF-2 activity²¹ and would have to be removed very efficiently in vivo before the initiation factor could catalyse another round of protein synthesis. It is not known how this is achieved in the intact cell, but in vitro eIF-2 activity is enhanced by energy-generating systems such as phosphoenol pyruvate/pyruvate kinase¹⁷, phosphocreatine/creatine kinase²² and nucleoside diphosphate kinase²¹ (which rephosphorylate free GDP), or an enzyme which hydrolyses GDP to GMP and phosphate¹⁷. We have now developed model systems intended to reflect the process of guanine nucleotide exchange on eIF-2 in vivo and have investigated the effects on this process of phosphorylation of eIF-2 by HCR in vitro.

GDP displacement from eIF-2 was assayed by incubating eIF-2 with this nucleotide in a three stage experiment. The first stage consists of binding radiolabelled GDP to the initiation factor; binding is complete within 10-15 min at 30 °C, as measured by retention of radioactivity on cellulose nitrate filters. In the second stage the [eIF-2-GDP] complex is incubated with or without HCR and ATP. Finally, the bound GDP is displaced by adding excess unlabelled GTP (with or without initiator Met-tRNA, or GDP and the kinetics of nucleotide exchange are measured. Figure 1 shows that phosphorylation of eIF-2 after incubation with ³H-GDP has no initial effect on the amount of radioactivity already bound to the factor. The [eIF-2·GDP] complexes are also stable on further incubation (Fig. 1b). However, when unlabelled GTP or GDP is added, the labelled nucleotide is displaced with kinetics which depend on the state of phosphorylation of the factor (Fig. 1a, c). As shown in Fig. 1a, the initiation factor exchanges bound ³H-GDP for non-radioactive GTP, in the presence of MettRNA_f, over a period of 15-25 min. After treatment with HCR the [eIF-2·GDP] complexes are much more stable and do not readily exchange with GTP. In the absence of Met-tRNA, the behaviour of the [eIF-2.3H-GDP] complexes is very similar (Table 1). Phosphorylation of eIF-2 with HCR also prolongs the half life of [eIF-2.3H-GDP] complexes during exchange with a sevenfold molar excess of unlabelled GDP (Fig. 1c, Table 1).

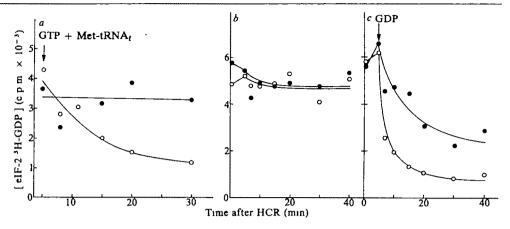
Experiments in which eIF-2 is incubated with HCR and $[\gamma^{-32}P]ATP$ and then analysed by SDS-gel electrophoresis

Table 1 Effects of HCR treatment on displacement of ³H-GDP from eIF-2

Expt	A 10 min	dditions at:	³ H-GDP bo 10 min 2 (c.p.m.×	5 min	% ³ H-GDP remaining at 25 min
1		CTD		1	
1	*****	GTP	4.24	1.62	38
	HCR	GTP	3.61	2.95	82
2		GTP	3.89	2.06	53
	HCR	GTP	3.59	3.78	105
		GTP+Met-tRNA,	4.31	2.00	46
	HCR	GTP+Met-tRNA,	3.65	3.17	87
3		GDP	2.69	0.80	30
	HCR	GDP	3,60	1.89	53
4	-	GDP	5.83	1.34	23
	HCR	GDP	5.60	4.48	80

The data summarize the results of several experiments carried out as described in Fig. 1 legend. Initiation factor eIF-2 was incubated for 10 min at 30 °C with ³H-GDP in the presence of 0.2 mM ATP; HCR (8 U ml⁻¹) was then added where indicated for a further 5 min. At this time the bound 3H-GDP was displaced from eIF-2 by addition of: expt 1, 2 μ M GTP (freshly prepared); expt 2, 50 μ M GTP (free of contaminating GDP), with or without 12.3 nM unlabelled Met-tRNA; expts 3 and 4, 2 µM unlabelled GDP.

Fig. 1 Effects of HCR on displacement of ³H-GDP from complexes with eIF-2. Initiation factor eIF-2 was prepared from a 15-1 suspension culture of ascites tumour cells suspending pelleted ribosomes in 14 ml of high salt buffer (0.25 M sucrose, 0.005 M MOPS pH 7.6, 0.5 M KCl, 0 005 M Mg-acetate, 0.001 M dithiothrestol (DTT), 0.1 mM EDTA) and re-centrifuging at 147,000g for 2 h. The supernatant (salt wash) was fractionated by ammonium sulphate pre-cipitation between 30% and 70% saturation, dissolved in 8 ml of 0.02 M MOPS, 0.35 M KCl, 0.5 mM DTT, 0.1 mM EDTA, dialysed overnight against this buffer and then subjected to chromatography on phosphocellulose (Whatman P11; 16×42mm column).



The column was washed with the above buffer, the eIF-2 was eluted with a 50-ml gradient of 0.35-0.8 M KCl in the above buffer and activity was detected by ternary complex formation (carried out in the conditions described in ref. 17). The fractions containing activity (eluting at 0.56-0.7 M KCl) were pooled, dialysed for 1 h against 0.05 M KCl in buffer, diluted with an equal volume of buffer without KCl and applied to a 3.5-ml column of DEAE-cellulose (Whatman DE-52) This column was washed with 0.09 M KCl in buffer, and eIF-2 was eluted with a 25-ml gradient of 0.09-0.4 M KCl in buffer and assayed as above. A peak containing 80% of the activity was pooled and precipitated by dialysis against 70% saturated ammonium sulphate in 0.02 M MOPS, pH 7.4. It was dissolved in 1 ml of buffer containing 0.09 M KCl and further dialysed against the latter for 1 h. The factor preparation, which was 30-50% pure, was stored in small aliquots in liquid mitrogen. The GDP displacement assay was carried out in three stages (1) ³H-GDP (6.7 µCl ml⁻¹, 0.47 µM m a; 5 µCl ml⁻¹, 0.35 µM in b and c) was incubated with eIF-2 (~12 µg of protein) in 150 or 200 µl for 10 mm at 30 °C in the presence of 0 02 M HEPES pH 7.6, 0.1 M KCl, 0.002 M Mg-acetate, 0.5 mM DTT, 0.1 mM EDTA and 0.2 mM ATP. (2) The incubation was then continued for a further 5 min in the presence of 8 U ml⁻¹ of partially purified HCR (1 unit gives 75% inhibition of protein synthesis in a reticulocyte lysate after 60 min). (3) Additions were then made of 50 µM GTP plus 12.3 pmol ml⁻¹ unlabelled Met-tRNA_t (a), nothing (b) or 2 µM unlabelled GDP (c) At the times shown, 20-µl samples were withdrawn, diluted in 1 ml of cold wash buffer (0.02 M HEPES pH 7 6, 0.1 M KCl, 0.002 M Mg acetate) and immediately filtered on 0.45 µm cellulose ester filters (Millipore). The filters were washed with 3×3 ml of wash buffer, dried and the bound radioactivity determined. a, Kinetics of dissociation of [eIF-2-³H-GDP] complexes in the presence of unlabelled GDP. O, Control eIF-2; M-

show that the presence of bound GDP, GTP or Met-tRNA_f does not affect the extent of phosphorylation of the factor in the conditions of the assays described in Fig. 1 and Table 1 legends (data not shown). Furthermore, no net phosphate is lost from eIF-2 during the longer third stage of the incubations. The data presented in Table 2 show that inhibition of guanine nucleotide exchange requires not only HCR but also ATP, and the latter must be present in a hydrolysable form. When ATP is absent or is replaced by its non-hydrolysable analogue, β , γ -methylene adenosine triphosphate, GDP displacement by GTP is unaffected by HCR treatment. These results are consistent with a need for HCR-catalysed phosphorylation of eIF-2.

A potentially significant aspect of this work is that these results have been obtained with preparations of eIF-2 which are not completely homogeneous. SDS-gel electrophoresis indicates, in addition to the major eIF-2 bands, the presence of several polypeptide components with molecular weights from 47,000 to 97,000 (data not shown). Furthermore, similar experiments using highly purified (homogeneous) eIF-2 show slow GDP exchange kinetics whether or not the factor has been phosphorylated. These results suggest that nucleotide exchange may be catalysed by another component, analogous in its action to the polypeptide elongation factor EF-Ts²³⁻²⁵. component may be present in our partially purified eIF-2 but absent from the homogeneous factor, and the interaction between this component and eIF-2 could be sensitive to the state of phosphorylation of the latter. Several laboratories have reported the existence of one or more accessory proteins²⁴ which stimulate eIF-2 activity when the initiation factor is assayed at low (physiological) levels in the presence of Mg2+, and which interact with the unmodified but not the phosphorylated form of eIF-2^{26,28-30}. Recently, a very similar factor has been characterized in the ribosome wash from Ehrlich cells and procedures have been developed for its separation from eIF-2 (L. R. V. Panniers and E.C.H., unpublished data). We are now therefore investigating the possibility that this factor acts by catalysing guanine nucleotide exchange on unmodified, but not phosphorylated, eIF-2.

During this work we have observed that not only the dissociation of GDP but also the binding of this ligand to our eIF-2 preparations is apparently inhibited by phosphorylation of the initiation factor (Fig. 2). As with the exchange reaction, the effect is totally dependent on the presence of both HCR and

ATP and is therefore unlikely to be an artefact caused by any GDP-binding or degradative activity of the HCR preparation used. There is evidence that the α subunit of eIF-2 is responsible for GTP and GDP binding¹⁸, so it is possible that incorporation of a phosphate group on this polypeptide interferes with both the entry and exit of GDP at the nucleotide binding site. Both processes could involve the putative guanine nucleotide exchange protein. Alternatively, the possible presence of unlabelled GDP already bound to the eIF-2 could prevent the association of ³H-GDP with the factor, due to inhibition of

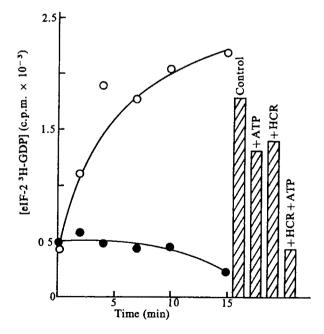


Fig. 2 Inhibition of GDP binding to eIF-2 by HCR-catalysed phosphorylation. The kinetics of binding of $^3\text{H-GDP}$ to eIF-2 were measured using the conditions described in Fig. 1 legend. Half the amount of eIF-2 was used and the initiation factor was incubated with 8 U ml $^{-1}$ HCR and 0.2 mM ATP, where indicated, for 5 min before addition of the $^3\text{H-GDP}$ at time zero. Samples (20 µl) were withdrawn at times shown for assessment of $^3\text{H-GDP}$ binding as described in Fig. 1 legend. O, Control eIF-2, \spadesuit , eIF-2 treated with HCR and ATP. The histogram shows the requirement for both HCR and ATP in the inhibition of GDP binding, measured after 7 min of incubation.

Table 2 Requirement for ATP hydrolysis for inhibition of nucleotide exchange

Additions	³ H-GDP 15 min (c.p.m	% ³ H-GDP remaining at 30 min	
ATP	7.45	1.17	16
ATP+HCR	8.25	3.42	41
Glucose +			
hexokinase	8.48	1.35	16
Glucose + hexokinase + HCR	8.65	1.08	12
AMPPcP	8.06	1.04	13
AMPPcP+HCR	9.02	1.85	21

Incubations were carried out as described in Fig. 1 legend, except that eIF-2 was incubated for 15 min at 30 °C with ³H-GDP in the presence or absence of 0.2 mM ATP or its non-hydrolysable analogue, AMPPcP, or of 10 mM glucose + 10 U ml-1 hexokinase (to remove any traces of ATP present in other components). HCR was then added where indicated for a further 5 min, at which time the bound ³H-GDP was displaced by addition of 50 µM GTP+12.3 nM unlabelled Met-tRNA_t. The data show the ³H-GDP bound to eIF-2 in 30 -µl samples removed at 15 min (immediately before HCR addition) and 30 min (10 min after GDP and Met-tRNA, had been added). Virtually identical data were obtained when ATP or glucose + hexokinase were added at 15 min rather than at zero time (not shown).

exchange after phosphorylation. The relatively slow kinetics of ³H-GDP binding to non-phosphorylated eIF-2 are consistent with such a possibility, although it is not known whether [eIF-2.GDP] complexes would remain stable through the several stages of purification involved in the preparation of the initiation factor.

In conclusion, we propose that phosphorylation of initiation factor eIF-2 inhibits the rate of initiation of protein synthesis by slowing the kinetics of exchange of GTP for GDP generated during 80S initiation complex formation. Although the results described here all concern the effects of phosphorylation catalysed by the haem-regulated protein kinase HCR, because the same site on eIF- 2α is phosphorylated by the reticulocyte double-stranded RNA-activated protein kinase, and by a similar enzyme induced in numerous eukarvotic cell types by interferons, it seems probable that protein synthesis may be similarly regulated at the level of initiation in these systems.

We thank Vivienne Tilleray and Eileen Canfield for technical assistance and Dr Tim Hunt for a gift of purified HCR. This work has been supported by USPHS grant CA-21663 to E.C.H. M.J.C. is the recipient of a Career Development Award from the Cancer Research Campaign. E.C.H. received a Wellcome Research Travel Grant for collaboration on this project.

Received 27 August 1981; accepted 4 January 1982.

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Maternally transmitted factors modify development and malignancy of teratomas in mice

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Maternal effects on the genome of the developing embryo have been well documented for amphibians^{1,2}. The existence of a similar maternal effect transmitted by the cytoplasm of the mammalian ovum has also been postulated but the evidence for this remains controversial and only one system, which demonstrates these maternal effects on development in the mouse, has been reported4.5. Mouse embryos (7-day-old) transplanted under the kidney capsule of adult isogeneic recipients give rise to either benign or malignant teratocarcinomas6, the yield of malignant tumours being high in some inbred strains but low in others'. In teratocarcinoma(TC)-permissive strains, such as C3H/He (C3H), or BALB/c, 50-70% of all embryo-derived tumours are malignant7, compared with only 10% in TC nonpermissive strains, such as C57BL/6. This genetic non-permissiveness is not absolute and we have shown that the outcome of embryonic transplantations depends on both the embryo and the adult recipient of the graft⁶. We now present evidence that in reciprocal F₁ hybrids, TC permissiveness is determined by maternally transmitted factors. As these matroclinous differences were evident during early stages of embryonic development when the embryo had only briefly been in contact with the uterus, we believe that the differences are due to cytoplasmic factors transmitted in the ovum rather than metabolic maternal uterine effects.

We compared the weight of tumours and the ratio of teratomas to teratocarcinomas obtained from 7-day-old mouse egg cylinders of different derivation, transplanted into isogeneic or F₁ adult recipients. The experiments were performed using embryos of two TC-permissive strains (C3H and BALB/c), one TC non-permissive strain (C57BL/6) and F₁ embryos obtained by reciprocal mating of TC-permissive and non-permissive parents. The embryos were transplanted under the kidney capsule of histocompatible female adult recipients and the tumours obtained from the embryos were collected after 2 months, weighed and histologically classified as either benign teratoma or malignant teratocarcinoma.

In the first set of experiments, we transplanted inbred embryos to either isogeneic or histocompatible F1 recipients (Table 1). The F₁ hybrids were produced by reciprocal matings to obtain recipient animals whose mother or father belonged to either a TC-permissive or non-permissive strain. The tumours obtained from transplants to isogeneic and F₁ recipient animals were histologically similar but the ratio of benign to malignant tumours varied among different experimental groups. Table 1 shows that the permissiveness of C3H and BALB/c embryos was not altered on transplantation into F₁ hybrids and the ratio of teratomas to teratocarcinomas obtained from these embryos in F₁ hybrid recipients was essentially the same as in isogeneic transplantations. In contrast, the weight of tumours obtained from both C3H (except group 4) and BALB/c embryos transplanted to F₁ hybrids was greater than that obtained in isogeneic

Table 1 Percentage of malignant tumours obtained from 7-day-old inbred embryos transplanted under the kidney capsule of isogeneic and F1 hybrid adult recipients

Group	Embryo	Recipient	No. of grafts	Mean tumour weight (mg; ±s.e.m.)	Teratoma/ teratocarcinoma	%Of malignant tumours
1	СЗН	СЗН	29	3.822 ± 460	15/14	48
2	C57BL/6	C57BL/6	30	$1,319 \pm 308$	27/3	10
3	BALB/c	BALB/c	25	$2,312 \pm 210$	9/16	64
4	СЗН	(C57BL/6×C3H)F,	37	$3,410 \pm 695$	18/20	54
5	C3H	$(C3H \times C57BL/6)F_1$	40	5.436 ± 810	20/20	50
6	C57BL/6	$(C57BL/6\times C3H)F$	49	2.714 ± 540	39/10	20
7	C57BL/6	$(C3H \times C57BL/6)F_1$	31	4.212 ± 781	15/16	48
8	BALB/c	(BALB/c×C57BL/6)F,	29	5,666 ± 682	6/23	80
9	BALB/c	(C57BL/6×BALB/c)F ₁	27	$3,780 \pm 470$	3/24	89
10	C57BL/6	$(C57BL/6 \times BALB/c)F_1$	28	$1,740 \pm 380$	24/4	14
11	C57BL/6	$(BALB/c \times C57BL/6)F_1$	34	680 ± 240	30/4	12

Inbred mice C3H/He (C3H), C57BL/6 and BALB/c, and their F1 female hybrids were obtained from Jackson Laboratories or produced in our animal colony. In F1 hybrid mice, the female parental strain is shown first. The embryos were obtained from dated pregnancies. An embryonic portion of 7-day-old embryos was isolated and transplanted under the kidney capsule of adult isogeneic or F₁ recipients. Recipient animals were killed 2 months after grafting and the tumours were weighed. Diagnosis of treatoma or teratocarcinoma was made histologically. Probability values for weight of tumours as calculated by Student's 1-test were P < 0.005 (group 1 compared with 2, 2/7, 2/3 and 3/8); P < 0.02 (10 compared with 11); P < 0.05 (8/9, 1/5, 4/5). Probability values for the ratio of teratoma to teratocarcinoma, as calculated by χ^2 test were P < 0.005 (2 compared with 7, 7/10, 7/11); P < 0.01 (6/7); P < 0.05 (10/11); P < 0.2 (2/6).

Table 2 Percentage of malignant tumours obtained from 7-day-old F1 hybrid embryos transplanted under the kidney of histocompatible F1 hybrid adult recipients

Group	Embryo (F ₁ hybrid)	Recipient (F ₁ hybrid)	No. of grafts	Mean tumour weight (mg; ±s.e.m.)	Teratoma/ teratocarcinoma	% Of malignant tumours
1	C57BL/6×C3H	C57BL/6×C3H	35	$1,469 \pm 433$	29/16	17
2	C3H×C57BL/6	C57BL/6×C3H	38	4.072 ± 850	20/18	47
3	BALB/c×C57BL/6	BALB/c×C57BL/6	29	$5,686 \pm 1,179$	14/15	52
4	C57BL/6×BALB/c	BALB/c×C57BL/6	24	$1,460 \pm 776$	22/2	8

In F, hybrid mice, the maternal strain is shown first. Probability values for weight of tumours as calculated by Student's t-test were: P < 0.005 (1 compared with 2, 3 with 4). Probability values for the ratio of teratoma to teratocarcinoma as calculated by χ^2 test were: P < 0.001 (3 compared with 4); P < 0.01 (1 compared with 2).

transplantations. The non-permissiveness of C57BL/6 embryos as evidenced in isogeneic transplantations (group 2) was abrogated in transplantation to some F₁ hybrids (group 7) but not to others (groups 10 and 11). Note that the ratio of teratomas to teratocarcinomas obtained from C57BL/6 embryos increased significantly on transplantation to (C3H×C57BL/6)F₁ hybrids whose mother was TC permissive (C3H) but did not increase in reciprocal (C57BL/6×C3H)F₁ hybrids whose mother was TC non-permissive (C57BL/6). Paradoxically, the weight of tumours obtained from C57BL/6 embryos was lower in (BALB/c×C57BL/6)F₁ hybrids than in either isogeneic or $(C57BL/6 \times BALB/c)F_1$ recipients, although the ratio of malignant to benign tumours did not change. These data indicate that the TC non-permissiveness is relative and may be modified by the host. It is also obvious that in reciprocal hybrids the malignancy of embryo-derived tumours is determined by the parental derivation of the recipients. The increased yield of malignant tumours and accelerated growth of tumours occurred only in the F₁ hybrids whose maternal strain was TC permissive (C3H).

In the second set of experiments, we compared weight of tumours and the ratio of malignant to benign teratomas obtained after transplantation of F₁ hybrid embryos to histocompatible F₁ recipients derived from matings of TC-nonpermissive C57BL/6 strain and the TC-permissive strains (C3H and BALB/c). In each combination, the materno-paternal strain derivation of recipient animals was kept constant and the derivation of embryos was reciprocal. Table 2 shows that the embryos whose maternal strain was TC non-permissive (C57BL/6) gave rise to teratocarcinomas in essentially the same ratio as the inbred C57BL/6 embryos. In contrast, the embryos whose mothers were TC permissive and the fathers TC nonpermissive $((C3H \times C57BL/6)F_1 \text{ and } (BALB/c \times C57BL/6)F_1$ embryos) gave rise to significantly more teratocarcinomas than the reciprocal hybrid embryos. The ratio of teratoma to teratocarcinoma in the two latter groups corresponded to the ratio expected from isogeneic embryo transplants of C3H and BALB/c (maternal strain) embryos.

The F₁ adult recipients and embryos produced by reciprocal mating of a TC-non-permissive and two TC-permissive strains have identical genomes notwithstanding the Y chromosome

present in ~50% of the embryos. Nevertheless, the outcome of embryonic transplantations was markedly influenced by the maternal strain of the F₁ hybrid recipient animals and even more so by the derivation of F₁ hybrid embryos. These differences indicate a matroclinous effect, but additional studies are needed to determine whether this effect is due to the maternal environment during embryonic development (the 'uterine effect') or 'cytoplasmic factor(s)' transmitted in the cytoplasm of the ovum³. The present data do not rule out either of these hypotheses. The experiments with F₁ hybrid embryos indicate that the differences between reciprocal hybrids are already evident in the early postimplantation stages of embryonic development, thus favouring the existence of cytoplasmic factors, as it is unlikely that external, maternal metabolic influences could have modified so profoundly the developing embryo, as it had only briefly been in contact with the maternal organism before transplantation.

This work was supported by USPHS grants CA23097, CA10815, CA21069 and GM29040.

Received 4 September 1981; accepted 15 January 1982.

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Errata

In the letter 'T-cell mitogens cause early changes in cytoplasmic free Ca²⁺ and membrane potential in lymphocytes' by R. Y. Tsien, T. Pozzan and T. J. Rink, Nature 295, 68-71 (1982), on page 69 the concentration of the phosphodiesterase inhibitor Ro20/1724, should be 10 µM and that of dibutyryl cyclic AMP should be 0.25 mM.

In the letter 'Atmospheric angular momentum and the length of day: a common fluctuation with a period near 50 days' by R. B. Langley et al., Nature 294, 730-732 (1981), on page 732 the acknowledgement should read that R.B.L. was supported in part by a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship.

BOOK REVIEWS

An inspection of inspectorates

Eric Ashby

A BOOK written under the surveillance of a committee, setting out the results of a project supported by a grant from the Social Science Research Council: surely the kiss of rejection for book-reviewers! Perhaps that is why this book has received so little attention. I am glad to have the chance to redeem this neglect. For the book does not have that bland anonymity associated with such multiple parentage. The committee was a working group set up by the Royal Institute of Public Administration to assist Mr Rhodes in his research; but they left Mr Rhodes on his own to write the book and it has the imprint of a man of independent mind examining the material he has collected and coming to clear and well-argued conclusions about it. The conclusions deserve careful study and further discussion.

At the end of his book Mr Rhodes asks (though not as bluntly as this) "Are inspectors really necessary?". He explains how difficult it is to get an answer to the antecedent question: "What good do they do?", and he shows how one needs to dig into their history in order to appreciate their present usefulness. There are two kinds of inspectorates; those that have to ensure compliance with statutory requirements (enforcement inspection) and those that have to maintain or improve standards of performance (efficiency inspection). At extreme ends of the scale of inspectorates there are clear differences between these two categories: an inspector of weights and measures has to enforce the law that grams and litres used in trade are to be uniform all over the country; an inspector of schools does not have to enforce compliance with any statutory standard. But - and this is what makes the topic of inspectorates so complex and so controversial - history demonstrates that the most effective enforcement inspectorates do not depend for their efficacy on enforcement (in the literal sense). They do have the power to bring culprits to court, and on paper it may seem that their job is to act like policemen enforcing a 30-mile-an-hour speed limit. But that is not the way they work. Even factory and mines inspectors, up against bitter opposition from mill and mine owners who resented interference from the state, learnt early in their history that they got the best result from being (as the chief inspector of factories in 1878 put it) "the friend of the manufacturer as much as the friend of the employé". The first alkali inspector, charged in 1863 to enforce a 95 per cent reduction in the emission of hydroInspectorates in British Government: Law Enforcement and Standards of Efficiency. By Gerald Rhodes. Pp.281. ISBN 0-04-351056-6. (George Allen & Unwin: 1981.) £15, \$35.

chloric acid from soda works, came to the same conclusion: I work, he wrote, "at present, by advice and friendly admonition, and prosecutions will come in their proper time".

Except for flagrant and defiant offenders, the time for prosecutions never has come. This is in striking contrast to the style of administration in the United States. In the control of pollution, for example, there are at any one time hundreds of cases in the courts in the USA, compared with five or ten at most in Britain. What is the reason for this difference? One reason is that inspectorates in Britain have acquired, in over a century of practice, a discretion to make what are really quasi-political decisions, using their judgement in the way they interpret their powers under the law. There is no provision in law (for instance) for an obsolete cement works, soon to be closing down, to be allowed a greater licence to pollute than a new cement works, just being put into operation. If the alkali inspector had no more discretion than a policeman, he could not allow such licence. But - as Mr Rhodes cogently argues in his book - it is just because the alkali inspector (and the same holds for other sorts of enforcement inspectorates) has been allowed discretion that his work is, on the whole, so effective in the public interest. Inspectors who use their discretion in such ways as this are, of course, vulnerable to the criticism that they are in the pocket of the manufacturer. But on balance this tradition in British inspectorates — a discretion to compromise and bargain cooperatively with the persons being inspected — has stood the test of a century of practice.

"Are inspectors really necessary?". Of course Mr Rhodes does not answer this question, but he leaves the reader with a pretty persuasive hint that he would say: "For the present, yes". Inspectors for efficiency, in particular, help to keep standards up to the mark; and even though there is no reliable way to measure their value quantitatively, the fact that they are highly valued by the very people whom they inspect is sufficient to justify their maintenance. Everyone who is liable to be inspected (and I guess that covers most

readers of *Nature*) should find time to read this admirable book. It explains how a race of officials who in some countries are the object of denigration, have — thanks largely to the perception and tact of their Victorian predecessors — become in Britain the object of respect.

Lord Ashby was formerly Master of Clare College, Cambridge. His most recent book, written with Mary Anderson, is The Politics of Clean Air (Clarendon/Oxford University Press, 1981.)

Sense of déjà vu

H.F. Rowell

Sense Organs. Edited by M.S. Laverack and D.J. Cosens. Pp. 394. ISBN 0-216-91094-3. (Blackie: 1981.) £29.75.

THE editors of Sense Organs state their object to be to produce

a set of stimulating contributions covering what is known about how biological sensors work, their sensitivities, their ambiguities, their input to the CNS and the manner in which their signals are interpreted.

Their attempt at this enormous task amounts to less than 400 pages, and not surprisingly the result is gappy and uneven. The book consists of papers presented to the Scottish Electrophysiological Society in 1980. The contributors appear to have been drawn from those in the field who were available in Britain or Western Europe at the time, and one suspects that the immense scope aimed for by the editors was determined by the diversity of the contributors, rather than the reverse.

The patchiness of the contents is accentuated by irrational ordering. For example, an article on behavioural correlates of photoreception is placed under "Structure and Function of Sense Organs", and within each of the book's two subsections there is no grouping of articles by either modality, phylum or level of analysis. Several contributions are traditional in style: the Introduction and the Epilogue by Autrum and Loewenstein, respectively, an article on sensory coding by Bullock, one on cognition by Gregory, one on "unusual" (i.e. speculative) senses by Brown, and a general essay on modalities by one of the editors. These or their near equivalents have appeared at most

symposia in this area for the past 20 years. The remainder are of two types: general reviews of a sub-field (thus Boekh on chemoreceptors, Sandeman equilibrium systems in arthropods, or Hawkins and Korner on the acousticolateralis system, to mention three of the best), or descriptions of the author's own work. Some articles which at first appear to be reviews are actually of the second type. Of the accounts of individual work, some of the best have already, and deservedly, had extensive public exposure (for example Land on a phylogenetic dichotomy in the optics of Crustacea, and Kirschfeld on the role of photostable pigments in vision) and produce a sense of déjà vu when encountered again with the long latency of a symposium volume.

However, I found three articles stimulating. Young gives a fascinating account of his research on the bizarre eye of Daphnia, and produces biologically satisfying explanations for many of its peculiarities. Light from above appears to be recognized by its high proportion of blue, side light in alga-rich water by green colour: appropriately sensitive and spatially organized receptors coupled with suitable behaviour enable the animal to stay the right way up and near to the surface, and to find and exploit algal patches. The author also convinces one that the "tremor" characteristic of this and similar eyes is unrelated to the interommatidial angle and hence not directly subserving scanning. Stieve reviews the role of calcium in visual transduction in invertebrates, and den Otter transduction in chemoreceptors. Both accounts are lucid and interesting and include much that is unfamiliar to me. As a neuron - rather than a receptor - physiologist I found the variety of off-beat mechanisms considered striking. Thus the control of light-activated channels in photoreceptors is attributed to a Ca/Na antagonism with competition for identical binding sites, and the possibility is entertained that the receptor potential of chemoreceptors may communicate with the spike initiating zone not electrotonically but via conformational change of microtubules or even via compression waves in the plane of the cell membrane.

I have slowly come to the view that the published symposium is the least useful form of scientific publication, except possibly for the isolated graduate student in a forgotten university. In general it consists only of material more adequately treated either in research articles or in review volumes. Most of Sense Organs reinforces this opinion — the useful bibliographies and the few articles which, as the editors put it, genuinely "convey the fascination and excitement of the topic" seem barely worth the tedium of the remainder.

H.F. Rowell is Professor of Zoology and Chairman of the Zoologisches Institut, University of Basel.

Astronomy: the politics and engineering

C.M. Humphries

Telescopes for the 1980s. Edited by G. Burbidge and A. Hewitt. Pp.278. ISBN 0-8243-2902-3. (Annual Reviews, Palo Alto, California: 1981.) \$27 (US), \$28 (elsewhere).

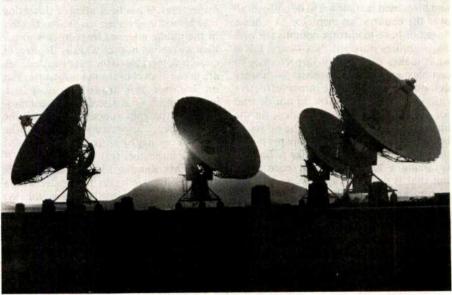
Not least among the ingredients required to bring a major national project from concept to fruition is perseverance. Budget trimming due to inflation, committee wrangles, procrastination and politics are just some of the adversities which may have to be weathered and which are all too familiar it seems, judging by the accounts of four large astronomy projects recently undertaken in the United States. Two of the projects described in *Telescopes for the 1980s* are ground-based telescopes and two are space observatories; together, they span the spectral region from radio frequencies to X-rays.

The Very Large Array was conceived in the early 1960s when it was recognized that existing radiotelescopes in the USA were incapable of extending extragalactic studies much further. Spurred by the need to create an imaging instrument with improved sensitivity and resolution, the last of the 28 movable 25m dishes (27 + 1 spare) was completed 18 years after the initial design studies began. In his account of the project, D.S. Heeschen comments that "in spite of real problems, petty irritations, miscellaneous distractions and excessive inflation, VLA construction proceeded pretty much on schedule and within budget". The result is a powerful aperture synthesis telescope with 351 separate baselines and an angular resolution of 0.1-2 arcsec depending on the wavelength band used.

The Multiple Mirror Telescope, described by authors from the participating groups at Tucson and the Smithsonian Astrophysical Observatory, is the subject of the next section. By comparison with the others, the MMT programme seems to have benefited by having a fairly loose organizational structure in its early years. Personal recollections by R.J. Weymann, former Director of Steward Observatory, give a nice insight into how that collaborative structure evolved and why it worked. On the matter of funding, the project was severely criticized at one stage for having started before financial backing was fully identified. Weymann's response to this is that "given the particular set of circumstances surrounding the MMT, to wait until funding had been located would have been tantamount to abandoning the project". A highly innovative optical and IR telescope was the result, and one which has an exciting new potential for speckle interferometry with the individual mirrors co-phased.

C.R. O'Dell's description of the Space Telescope project gives a fascinating account of the way in which new programmes evolve within NASA and of the relationship between NASA, the Office of Management and Budget (part of the executive branch of government) and the House of Representatives appropriation subcommittee. At one stage the ST was deleted entirely from the approved NASA budget for the financial year 1975 ("since the costs exceeded the choke limit of Congress"). When the project was later reinstated, the budget was less than NASA had requested, forcing a reduction in aperture to 2.4m and a re-examination of the scientific performance goals. Despite the compromises which had to be made, the 0.1 arcsec imaging capability still promises a huge observational advance.

The Einstein space observatory,



The Very Large Array — 27 mobile dishes, at maximum extension providing the equivalent of a radiotelescope 21 miles in diameter.

discussed in the final section by R. Giacconi et al., was launched in November 1978 and completed its operational lifetime 2½ years later. It, too, experienced some potentially disastrous setbacks (and had to be reduced in aperture by a factor of two) yet survived to make important X-ray observations with unprecedented sensitivity and an angular resolution (1–2 arcsec) which now matches that attainable with ground-based optical telescopes and the VLA.

Each of the sections in *Telescopes for the 1980s* contains a detailed account of the design and performance of the telescope and its instrumentation, and the sections on the VLA, MMT and Einstein observatory present examples of the scientific results obtained up to the end of 1980 (descriptions of the scientific uses of the ST are available elsewhere). The text is well supplied with diagrams and tabulated material, making a volume which is both useful and informative.

Colin Humphries is a Principal Scientific Officer at the Royal Observatory, Edinburgh, and was Project Manager for the construction of the United Kingdom Infrared Telescope.

Cinderella subject

P.G. Williamson

Paleoecology, Concepts and Applications. By J.R. Dodd and R.J. Stanton. Pp.544. ISBN 0-471-04171-8. (Wiley: 1981.) £29.55, \$53.15.

THE rennaissance that has swept large areas of palaeontology over the past two decades has left palaeoecology curiously untouched. New concepts in evolutionary palaeontology, such as punctuated equilibrium theory, are currently subjects of lively controversy within general evolutionary biology: palaeontologicallybased approaches to morphological analysis - such as Seilacher's "Konstructionsmorphologie" - are major influences on neontological interpretations of functional morphology. Palaeobiogeographical investigations have provided significant insights into the development and relationships of modern faunas. Increasingly, these various fields of palaeontological investigation have made significant contributions to their neontological sister-disciplines. Up to now, palaeoecology has been a sad Cinderella, absent from this intellectual ball. The main interest of this book is that it indicates — in part at least — why this is so.

The authors' avowed intent is to illustrate the utility of palaeoecology in palaeoenvironmental reconstruction. To this end, their book consists largely of a long series of anecdotal summaries of

previously published palaeoecological analyses. Chunks of basic ecological theory are periodically interjected; these are followed by further speculations as to how insights from modern ecology might, with varying degrees of ambivalence, assist palaeoecological reconstruction in particular instances. This approach has the unfortunate effect of revealing palaeoecology as a largely derivative field, a poor-man's applied ecology performed on inadequate data.

The dreary state of the discipline is best illustrated by those cases in which intensive work over several years has permitted the comprehensive characterization of given palaeoenvironments. A case in point is Stanton and Dodd's own exhaustive - and entirely admirable - work on the Pliocene Kettleman Hills deposits of the San Francisco area. This work is used as a connecting thread to link the various palaeoecological techniques explored in successive chapters (a good idea, incidentally). Assiduous application of various techniques over several years has allowed a detailed understanding of conditions in the Pliocene marine embayment of the Kettleman Hills. The upshot of all this effort seems to be that palaeoenvironments in the Kettleman Hills during the Pliocene were, well, rather as you might expect. Salinities varied from time to time and area to area. The biota varied correspondingly and predictably from time to time and area to area. This kind of empirical analysis could be repeated a million times in the hundreds of millions of cubic kilometres of the global Phanerozoic rock record, but hardly seems a potentially fruitful research strategy for a science aspiring to an original conceptual framework.

Admittedly, these are problems of the discipline as much as of the book. But the book itself suffers from serious shortcomings. These are sins of omission rather than commission, but are problems nonetheless. There is virtually no discussion of the plethora of statistical techniques that have so usefully been applied to specific palaeoecological problems in recent years. This is particularly unfortunate as the book claims to emphasize the applications of palaeoecology. To this extent — as a teaching aid — the book compares unfavourably with such texts as Reyment's Introduction to Quantitative Palaeoecology (Elsevier, 1971). Most discussion of numerical techniques is superficial: this supposedly graduate-level text provides no usable explanation of rarefaction! Discussion of techniques for the measurement of faunal diversity surely no small matter in palaeoecological studies - merits a scant four pages, and Fisher's parameter α isn't even mentioned. You do not have to be a rabid Hennigian to wonder why an entire chapter, supposedly devoted to palaeobiogeography, lacks any reference to vicariance biogeography.



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Stanton and Dodd provide a useful general survey of the palaeoecological literature. But this survey is rendered somewhat inaccessible by the fact that there is no author index. The book is overlong for what it has to say, and there is an unfortunate tendency towards Haigspeak: "Using the community... as an information transfer unit is most effective in the Neogene"! The book could be two-thirds of its length and still be an equivalent

"information transfer unit".

I suggested previously that palaeoecology has been the absent Cinderella at the modern palaeontological ball. Stanton and Dodd's book suggests that she may have some time to wait for the Good Fairy, never mind the glass slipper.

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Spot the potential carcinogen

Alastair Hav

Evaluation of Short-Term Tests for Carcinogens. Progress in Mutation Research, Vol.1. Edited by Frederick J. de Serres and John Ashby. Pp.827. ISBN 0-444-00570-6. (Elsevier/North-Holland: 1981.) Dfl.295, \$125. Short-Term Tests for Chemical Carcinogens. Edited by H.F. Stich and R.H.C. San. Pp.518. ISBN 3-540-90496-4. (Springer-Verlag: 1981.) DM 142, \$64.60.

CANCER caused by contact with chemicals at the workplace and in the environment is something regulatory authorities are trying to control. The task is a daunting one. New chemicals reaching the market place each year are numbered in the thousands. Yet each chemical must be tested to assess its toxicity, and in particular its potential for causing cancer. Can this be done using tests that will satisfy the regulators, but will not bankrupt industry? And, just as important, are there assays which will give an answer in days, as opposed to the two years for the standard animal carcinogenicity study?

There would seem to be an optimistic answer to both questions. There are tests which satisfy these criteria and which can be used to predict carcinogenic activity. The first of the so called "short-term tests" for carcinogens was originally developed by Dr Bruce Ames at the University of California (the Ames test). Using bacterial strains of Salmonella, Ames showed that chemicals which caused cancer in animals would cause bacteria to mutate. This ability to cause mutation, he argued, could be exploited to test for carcinogens. Thus a chemical which was mutagenic in the Salmonella test would be considered to be a potential carcinogen.

Unfortunately it is not that simple. Neither the Ames test nor the others developed since its discovery — based on the mutation of other bacterial, fungal or mammalian cells — will identify every potential carcinogen. The short-term tests are fickle. They give better results for one class of chemical than for another. In addition, many of the tests have been evaluated with the researcher knowing both the identity of the chemical being

tested and that it is carcinogenic in animals.

This kind of information is not going to be available when new chemicals are being tested. In this case, the tests will have to predict which chemicals are likely to cause cancer. Until recently short-term tests had never been evaluated under conditions in which they would be used by regulatory authorities to identify problem chemicals.

In 1976, however, a start was made to remedy this situation. Evaluation of Short-Term Tests for Carcinogens is the result of that initiative. It is the report of a unique international programme of collaboration between scientists in many countries to look at the ability of these tests to predict carcinogenicity. The venture, started by the UK Medical Research Council and Health and Safety Executive, was broadened to include scientists from ICI's Central Toxicology Laboratory and the US National Institute of Environmental Health Sciences. The US Environmental Protection Agency and the National Cancer Research Institute in Japan also made a financial contribution to the study.

Scientists in some 60 laboratories were sent 42 chemicals, 25 carcinogens and 17 non-carcinogens. The chemicals were coded and the identities were only revealed after the investigators had submitted their final results. According to the organizers, the chemicals were selected on the basis of the information available about their carcinogenicity in animals. Examples from most of the major classes of organic chemical carcinogens were chosen, as were chemicals which are either "vital to life or are considered essential to our modern lifestyle". This latter group included three chemicals assumed to be non-carcinogens such as methionine, vitamin C and household sugar.

Because of the design of the study, the 30 or so tests under scrutiny were being evaluated as predictors of carcinogenic activity and not just as tests to look at the mutagenic properties of chemicals. Thankfully, the results of this expensive but unique exercise in collaboration show that the tests have an important role to play in any programme to screen for potential carcinogens.

In the introductory chapters to this book the editors set out the reasons for the study; the basis for selection of chemicals used; the animal carcinogenicity data on these chemicals; and a summary of the results obtained by the laboratories. In the remaining 60 or so chapters each investigating team describes their own experimental protocol and discusses the results obtained in detail.

The book contains a wealth of material which will be of immense value to anyone interested in identifying chemicals which are potential carcinogens. The results of the study show that the most effective tests are those which use bacteria such as Salmonella or Escherichia coli. However, these bacterial tests do fail to pick out some potential carcinogens which other fungal or mammalian tests will identify. But these other tests in turn fail to identify chemicals which the bacteria pick out. All tests have false negatives and false positives and the study shows that a battery of tests will be required for satisfactory results.

The unequivocal evidence about a chemical's potential for causing cancer is always regarded as being provided by tests in animals; indeed this study was based on that premise. It is somewhat ironic, therefore, that the study has shown that animal carcinogenicity data are not always as good as they might be. Azoxybenzene is said to be a non-carcinogen in animals vet many of the in vitro tests identify it as a potential carcinogen. According to one of the editors, John Ashby, this suggests that the negative carcinogenicity data on this chemical which are available may not be reliable. It is recommended that azoxybenzene should be re-tested in animals.

Imaginative titles for books on screening tests for carcinogens are obviously in short supply. Neither of the two books reviewed here has what one might call an arresting label. But the titles do at least tell the reader what he or she is going to find. The second book, Short-Term Tests for Chemical Carcinogens, contains papers from 43 experts in the field, some of whom participated in the international study. The papers describe different tests in detail and the evaluation of some of them in individual laboratories.

The value of this second volume is in the scope it gives to authors to discuss the more neglected area of viral tests for carcinogens. And some authors address the complex issues of anticarcinogens, cocarcinogens, promoters, sensitizers and DNA repair inhibitors. The role these other factors play in the mechanism of carcinogenicity is still far from clear. However, with the research effort now being devoted to this area answers will not be long in coming. These two books show that the effort so far has been worthwhile, and that the short-term test has a vital part to play in detecting potential carcinogens.

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nature

11 March 1982

Prospect of sanity for oil prices

The weakening of oil prices in the past six months may — just may — presage the ending of the long recession. Will the oil consumers have the wit to see what they must do now?

Is the recent decrease of the international price of oil a sign that the great economic recession is about to end, or the first swallow whose appearance does not mean that summer has begun? Of necessity, there can be no quick answer. Only time can tell. But there are serious dangers in the arguments now going the rounds among economic soothsayers in the industrialized oil-consuming countries that rising prosperity is once more just around the corner. The most obvious risk is that people will now be tempted to falter in their pursuit of the strategies that have led to the steady reduction of the consumption of petroleum from the members of the Organization of Oil-Exporting Countries (OPEC), and thus to the decrease of prices in the past few months. The second risk, less obvious, is that the oil-consuming states will continue to under-estimate the need — and the opportunity — for making arrangements among themselves for buffering the cost of oil against the fluctuations decreed by OPEC and others.

That the weakening of oil prices could be a cheerful sign is beyond dispute. Just as successive increases of the international price of oil since 1973 helped to create the blend of price inflation and economic deflation that has hamstrung the oil-importing states, so a reduction of price should have the opposite effect less rapid inflation, more economic activity and people spending less on petroleum products and thus having more to spend on other things. The calculation is that a 10 per cent fall of oil prices should reduce the costs of goods and services by about one per cent and yet lead to an increase of demand in the oil-consuming states of about 0.5 per cent. The decrease in the price of oil in the past six months is more like 12.5 per cent than 10 per cent, but the economists are right to say that even the small reductions of prices and small increases of production that would result would feed on each other. In any case, the optimists argue, there may be further reductions of oil prices in the months ahead and so, with luck, a recovery of many industrialized economies.

Before throwing their hats in the air, the oil consumers should think harder than is their habit about what lies ahead. Although the decision by Saudi Arabia to reduce its oil production by 1 million barrels a day (50 million tonnes a year) will not directly affect the now weak international market for oil, it may ensure that the emergency meeting of OPEC provisionally arranged for 18 March is less incoherent than its immediate predecessors. For OPEC is far from dead, the jubilant obituaries of the past few weeks notwithstanding. But OPEC, commonly called a cartel. has yet to demonstrate that it can function at a time of falling demand for oil as a means of restricting output on a scale sufficient to influence the international price of oil. Its essential weakness is that many of its members have come to look to the whole of their oil revenues for their own economic support, with the result that they cannot with equanimity contemplate either a reduction of the price of oil or a reduction of their own exports. Apart from Saudi restraint, the only substantial restriction of exports that there has been so far has been caused by the determination of Iran and Iraq to do as much damage as they could to each other's oil installations as part of their private (and pointless) war. Yet even an agreement to a temporary reduction of exports, say for six months, could, if it were substantial, steady the falling price of oil and perhaps even make it rise again. The swallow that the industrialized oil importers have joyously been contemplating could quickly vanish.

More lasting economic forces within the oil-consuming states will also help to moderate the rosy prospect ahead. If the price of oil decreases, some plans for exploiting marginally economic reserves of oil and natural gas in the oil-consuming states will have to be postponed, moderating the rising trend of oil consumers' domestic production in the past few years. In any case, if industrial production does pick up again, so will the demand for oil. And if OPEC can find a basis for functioning as a true cartel. fixing the price and agreeing within itself not to break the rules even if exports should fall drastically as a result, its members' reduced demand for goods and services will partially offset the expected increase of economic activity among the oil consumers. So the economic recovery now possible is by no means a foregone conclusion. And to the extent that the oil consumers are by no means in charge of events, they will simply have to hope for the best in the next few weeks.

At the same time, they should recognize that whatever happens within OPEC will not bring back the circumstances of a decade ago, when the international price of oil was roughtly a tenth of what it has become. Not merely has much of the oil that used to be extracted from the Middle East at a cost of \$1 a barrel or less already been sold, but the arrangements that have been made in the oil-consuming states to increase domestic production (Britain, in the North Sea, for example) provide a floor below which the price of OPEC oil need not fall. OPEC members would more readily settle on a rational policy in the next few weeks if they were given some kind of assurance that, even in present circumstances, none of their customers is looking for a return to the prices of the 1950s. Ever since 1973, the oil consumers have set their faces against anything that might smack of joint negotiation on the international price of oil — and they have been justified by the events of the past few months. Now, though, when the boot is on the other foot, it might make sense that the oil consumers should break silence to give their suppliers an intelligent account of what the next decade holds.

More of the same will be the best recipe. Only now, a decade after the price of petroleum increased fivefold in as many months. have the benefits of conservation become apparent in the energy budgets of the oil consumers. Both in the United States and, to a lesser extent, in Western Europe, industrial users of energy have understandably led the way. They have been encouraged to invest in energy-saving equipment by the high costs of oil and of equivalent fuels. Japan, almost wholly dependent on imported fuel, has done even better - again for reasons that are entirely understandable. But what the experience of the past few years has shown is that energy conservation is necessarily a slow process, one in which the cost of energy is only one of several influences. The doubt now raised by the falling price of oil is that the continued hankering of some governments, that of the United States especially, to shield people from the true cost of energy for political reasons will take the edge off the incentive to energy conservation. (Exhortation will not do instead, for although a barrel of oil saved is exactly equivalent to a barrel of oil extracted from the ground, saving oil when it would be cheaper to burn it is an economic waste.) The trend, apparent in the past few years, is to replace oil by other sources of primary energy. The modest reduction of the price of oil should not, however, take the wind out of the sails of nuclear energy programmes, or even schemes

for turning coal into alcohol or sunlight into electricity, provided they are strictly economic.

There are also more subtle steps to take. The oil consumers should have been heartened by the demonstration provided in the past few years that even when a commodity is inherently as scarce as crude petroleum, it cannot be indefinitely insulated from market forces. If the real price is too great, people will use something else or will use less energy. And while prices can be kept artificially high for a short time, such a state of affairs cannot persist. What the oil consumers should ask themselves is whether this time of relative plenty in the supply of oil should not be used as an opportunity for making the international market in petroleum more effective. One snag is that the past decade has persuaded too many of them that their domestic supplies of oil, however small, should be reserved for domestic consumption. This is the spirit in which the United States Congress has in the past insisted that Alaskan oil should not be exported direct from Alaska, and in which the British Parliament has repeatedly asked that oil from the British sector of the North Sea should first be landed in the United Kingdom. The long-term interests of both countries would be better served, however, by making sure that this substantial quantity of oil could be used as a way of making a true market price for oil

Free broadcasting

The British government has been converted to telecommunications. The other side of the coin is freedom.

Technological developments, frequently subversive of accepted economic truths, are occasionally a challenge to social norms. The technique of in vitro fertilization as a means of producing children may be such a case. The new techniques of telecommunications are a more immediate challenge, especially where governments have been unwilling to acknowledge which way the wind has been blowing. This challenge will soon crop up in Britain, where the government announced last week that it would indeed take up the licence granted to it (and all other sovereign states) by the conference of the International Telecommunications Union in 1979, and launch a directbroadcasting satellite. By all accounts, this development is part of a recent and belated conversion of the British government to the belief that satellite communications have a brilliant future and, more surprisingly, to the view that it will be a social benefit if most British dwellings can be "cabled up" - linked to some broadband distribution system capable of handling more like forty than four distinct video signals, as at present.

The belief that the time has come when a choice of television signals may be a social benefit flies in the face of the entire history of British broadcasting. While the government's conversion (see page 105) seems to stem from a calculation that there is money to be made by manufacturing equipment that can also be sold elsewhere, what it has forgotten is that all its predecessors have fought hard for the notion of public service broadcasting, the Reithian concept that electromagnetic waves are inherently different from other kinds of communication in that they are best used for the transmission of seemly messages, educative ideally, if entertaining then, in the last resort, improving. The subliminal knowledge that the British Broadcasting Corporation is the world's outstanding practitioner of this art may have helped, as much as the soundness of its plans, to persuade the British government to give the coporation the first refusal of the licence to use two television channels on the first British direct broadcasting satellite in 1985.

That is the thin end of the wedge. The way technology has developed, television signals have become a kind of token contraband—if they exist, and people hanker after them, people will find some way of seeing the pictures that they represent. At the worst, they may have to buy a video-recorder and the cassettes to go with it. Or a domestic dish with the appropriate re-coding equipment. Either way, the new technology limits the degree to

which the authorities can control what people see or hear by means of broadcast signals. But how can this knowledge be squared with what successive British governments have been saying within living memory — that there must be central control not merely of the signals but of their content? Charters approved by parliament at present restrict what can be broadcast by both the British Broadcasting Corporation and its commercial counterparts. Both kinds of organizations, for example, are enjoined to strike a political balance in the content of what they broadcast and not to offend public taste. From time to time, they get into trouble, and questions are asked in the House of Commons. But this happens only infrequently. For the most part, all concerned are united behind the principle that broadcasting is a community service which, until the past few weeks, has even lent support to the doctrine put forward by the Annan committee three years ago that it would be wrong to sanction pay-television lest that should allow some people to see television programmes denied to others, that it should permit the broadcasting of indecencies and (under Labour governments) that it should allow commercial companies as yet unlicensed to make a profit.

That mask must now crack, although it is far from clear whether the present British government has accurately appreciated the difficulties it will face in converting a voting public from the present condition in which television signals are needlessly scarce to one in which all those who wish to send out broadcast signals, and who can pay the cost, will be allowed to do so. There will be fierce opposition from those who protest that a free-for-all will spell the end of quality in British broadcasting. (Less Shakespeare, no "Upstairs, Downtairs" or "Brideshead Revisited".) Sir Harold Wilson, once prime minister, will be only one of those who argue that providing more opportunities for would-be broadcasters will pave the way for what he called "cultural pollution" two years ago. Perhaps the British government is calculating that, with all the delay that there has been, the pent-up sense of opportunity among would-be investors in new broadcasting systems is so great that they will put up with the most restrictive fine print on whatever licences they are given.

The truth, however, is that even now, the British government is moving too slowly. And the accident that the International Telecommunications Union has allowed each sovereign state in the Old World the right to a single geosynchronous satellite is technically a red herring, an artificial spur to decision. For if a commodious cable system is economic for a country such as Britain, a single satellite will not be able to fill it with signals, which in any case could be distributed more economically in other ways. Satellites do, however, have the administrative advantage that it is possible to think of licensing those who wish to use them, while preventing those who wish to broadcast only by cable from putting their signals out. The trouble with such an arrangement is that it would be hopelessly uneconomic, and illiberal as well. It would be better that the British government should face at the outset the fact the creation of a national cable television system financed with private funds entails the abandonment of the old paternalism of the regulation of what is broadcast, however well that may have been accomplished.

For practical purposes, this is what has already happened in the United States. As on many other issues, Britain is being dragged along reluctantly, and a decade or so behind. As on previous occasions, the change when it comes will not be as traumatic as is feared. Elsewhere, however, things are even worse. In France, for example, the Mitterrand government, still flushed with its election victory, undertook to dismantle the apparatus of day-today control under which French broadcasting labours resentfully. Last July, the government's appointed committee suggested how this should be done. Now, the government seems to be having second thoughts. Governments that would not dream of censoring written communications plainly, but wrongly, think that electromagnetic signals are fair game for government licensors and inspectors. They should understand that they can no more embrace this new technology while keeping the old regulatory procedures than they could require horseless carriages to be preceded on the highways by a man with a red flag.

0028-0836/82/100102-01\$01.00

Legal shadow over laser patent

First court verdict for Gould claims

Washington

The simmering debate over who should be credited with the invention of the laser entered a new phase last week when a federal judge in San Francisco, spurred on by a New York technology investment company, upheld a patent issued to physicist Dr Gordon Gould as taking precedence over the original patent issued to Dr Charles Townes and Dr Arthur Schawlow in 1960.

Dr Townes shared the Nobel prize, with two Soviet physicists, in 1964 for his work in quantum electronics and microwave spectroscopy leading to the development of the laser or Light Amplification by Stimulated Emission of Radiation. Dr Schawlow shared the Nobel prize for his work as a chemist in the same area last year. Both are generally credited with putting forward the first conceptual design for a laser in a scientific paper published in *Physical Review* in December 1958 (112, 1940; 1958).

Dr Gould, however, who worked a few doors away from Dr Townes's laboratory at Columbia University in New York in 1957, claims that a notebook kept at the time demonstrates that he was the one to coin the word laser. He also claims that his conception, as described by his investment company backer, was that of an amplifying device, and should thus entitle him to credit for the conception of the laser as a whole.

It could not be established on Monday whether Dr Gould's claim extends to the basic process by which photons are amplified within a laser or to some other feature of the device. The substance of the claim appears to be the notion of creating a population of excited atoms, capable of stimulated emission, by exposing a gas or other material to an intense flash of light.

Dr Gould's claims for patent rights extending to an estimated 25-35 per cent of all lasers on the market, have been vigorously pursued by a New York-based company Refac Technology Development. With another company, Patlex of Pennsylvania, Refac has bought 80 per cent of any royalties generated by Dr Gould's patent for a sum claimed to be over \$2 million, and says it is now defending his claims to the laser against those of the scientific and technical establishment.

Until last week, the courts had been relatively unsympathetic. Dr Gould has failed several times to have revoked the patent granted to Townes and Schawlow in 1960, when both were working at Bell Laboratories, on the grounds that he had

been the first to conceive a complete working laser.

In 1977, however, the US Patent Office awarded Dr Gould a patent on his claim to have designed the amplifying device referred to in the patent. Since then, Refac has sought to obtain royalties from all companies producing optically-pumped lasers, and says that on the basis of a 1979 patent, also granted to Gould, it will now seek royalties on the use of such lasers.

Last week's decision, the first time that the validity of the patent has been tested in a federal court, was based on a suit filed by Refac against the Palo Alto-based company General Photonics claiming infringement of Gould's patent rights and demanding royalties on all lasers produced by the company since 1977.

Refac claims that Dr Gould should have received the technical — if not the scientific — credit for the first laser. The arguments

convinced Judge Samuel Conti and, as a result of his ruling in favour of Refac, General Photonics has agreed to pay the company 8 per cent of all its future sales.

Dr Arthur Schawlow, now professor of physics at Stanford University, has said that the US Patent Office was wrong to issue Dr Gould with the 1977 patent, and that Dr Townes "may have told Gould what he was doing" during conversations at Columbia in 1958. Dr Shawlow also says that drafts of the subsequent *Physical Review* paper were already circulating in the laboratory in August 1958, the date on which Gould wrote down some of his ideas.

Mr E.M. Lang, president of Refac, argues conversely that Gould spoke to Townes about his ideas on how to produce an amplifier that would make the laser work, and that it was Gould's ideas which were later incorporated into early laser designs for which Townes and Schawlow

UN university goes on tour

Paris, February

Global modellers, the inheritors of those who gave us *The Limits to Growth* in 1972, gathered here from 22 to 25 February to advise the Tokyo-based United Nations University on its preoccupation with the problems of agriculture, energy and development. The symposium, organized by Professor Maurice Lévy of the Marie and Pierre Curie University (otherwise Paris VI), seems not finally to have persuaded the university to take global modelling to its bosom, but the rector, the Indonesian Soedjatmako, and his four vice-rectors undertook to brood about the problem when they are back in Tokyo.

Part of the interest of the occasion was that it showed how stimulating modelling techniques of socio-economic problems have proved to be, if only as a spur to understanding how one variable is related to another. Thus a model of the rural economy of Bangladesh was widely acclaimed, but the work of the International Institute for Applied Systems Analysis, of the Organization for Economic Cooperation and Development and of Dr Sam Cole (University of Sussex) also received high marks.

Professor Donella Meadows, who has been working on a model of the rural economy of New England since her collaboration on the original Club of Rome report, argued passionately at the symposium for a kind of global network of global modellers, linked together by communications satellites and thus able to exchange information or computational codes easily. The United Nations University cautiously withheld its blessing.

One of the problems of such occasions is that the participants are in two camps —

those who would construct models that address some tangible aspect of a problem and those who hold that complexity is of the essence. Some of the most confusing (and energetic) contributions to the discussion came from those who argued that "techno-economic" models purporting to account for, say, the effects of fuel prices on food production, were certain to be inadequate, given their neglect of "socio-political" considerations.

The interest of the United Nations University in these studies stems from its wish partially to focus its interest on the encouragement of economic development on what the symposium called "the energy-agriculture nexus". Inevitably, in a gathering of systems analysts, some argued that "food" would be a better variable than "agriculture".

The United Nations University is not so much a university as the late U Thant's creation of a United Nations development agency. Its chief source of funds is a pledge of \$100 million from the government of Japan. The university has no students, while all its employees are on short-term contracts. According to Soedjatmako, negotiations are now under way for a permanent building in Tokyo, although this is unlikely to be built before the end of his term of office in 1985.

Lacking a permanent establishment, the university works chiefly by forming links with academic groups elsewhere, in industrialized and developing countries alike. It was encouraged, at the end of its four-day stand in Paris last month, to be told by the newly created minister of external affairs at the French Department of Education that French academics will be asked to collaborate with the university.

had been awarded the patent.

The scientific record is sufficiently ambiguous to allow for conflicting interpretations of the facts. Lang argues that the details of a proposed amplifier included in the Physical Review paper and the subsequent patent were shown not to work, and that Gould's ideas should therefore take precedence. Schawlow's response is that the existence of a working amplifier was implied in the paper, and that even though the specific solution suggested did not succeed, other lines of approach were suggested which proved successful. He also maintains that ideas about possible amplifiers were part of the "state of the art" at the time, and hence not eligible for patent protection on behalf of any one individual.

Refac is using last week's decision to bolster its claims on behalf of Gould. Its share price rose 12 per cent in value after the verdict had been announced. However, others are unconvinced; Dr Schawlow says that the case was poorly defended by General Photonics, which has already admitted that it does not have the money to mount an appeal.

More telling is likely to be a separate suit filed by Refac against Control Laser of Florida, a leading manufacturer of optical lasers. This suit was filed within a few days of the patent being granted in 1977, and has already attracted wide interest from other manufacturers (who once intended to join the suit in opposition to Refac, but then decided to withdraw for fear of being challenged on anti-trust grounds).

When the Control Laser case comes to trial, the company stands to lose a considerable amount of money if the verdict goes against it. Mr Robert van Roijen, the company's president, said last week that the major point of dispute was whether Refac's 1977 patent covered merely the optically-pumped amplifier described in the patent application, or whether — as Refac claimed — the patent could be taken to cover the whole apparatus.

Mr van Roijen would be willing to pay royalties on the amplifier, but denies that a laser patent is involved because "it would cost only a few thousand dollars". His arguments are expected to be backed by Dr T.H. Maiman, a director of Control Laser, who was the first to publish details of a working model of the laser (*Nature* 187, 493; 1960).

Looming on the horizon, however, is another suit which Refac has filed against a separate company for infringement of the "use" patent; in this case, General Motors has joined the proceedings on the side of the defendant.

Refac continues to characterize such disputes as a David-and-Goliath conflict. The companies maintain that Refac is using Gould's research to support a position that has been consistently rejected by the courts, and that the San Francisco verdict was, in Mr van Roijen's words, a "travesty" that is unlikely to survive the next legal round.

David Dickson

Pest research centres

Foreign labs shut

New Delh.

Accusations that the big powers conduct espionage or militarily oriented research under the guise of science collaboration in developing countries have again surfaced in the wake of the recent expulsion of an American scientist, Dr David R. Nalin, from Pakistan. The expulsion followed allegations that the United States aided Pakistan Medical Research Centre (PMRC) in Lahore which he headed was engaged in research on the use of mosquitoes in germ warfare. Six years ago another US funded mosquito control project in India was closed down by the government following similar allegations.

Dr Nalin denies the charge. In an interview he said the allegation was part of a Soviet smear campaign against the United States in retaliation against American accusations that the Soviet Union had indulged in germ warfare using mycotoxins in Kampuchea. Dr Nalin claims that his centre was infiltrated by left-wingers who organized strikes and spread rumours of a connection between PMRC and the Central Intelligence Agency. Nalin said that one member of his staff had been shown to have Soviet connections. He said

Expulsion denied

Washington

The Pakistani embassy in Washington denied last week that Dr Nalin had been expelled from the country because of the allegation over his involvement in bacteriological warfare research.

The Minister of Information at the embassy, Mr M.I. Butt, said that it had been decided not to renew Dr Nalin's two-year contract as director of the Medical Research Center in Lahore after it expired last August because of what he described as Dr Nalin's failure to stick to procedural requirements for administration and research, and tension with other members of the centre's staff which eventually led several of them to resign. However he added that Dr Nalin had been allowed to stay in Pakistan until the end of January in order to complete a report on his research.

Dr Nalin, speaking from the University of Maryland, said that the future of the research centre was now uncertain, since applications for renewed funding from the Agency for International Development and the National Institutes of Health had been disrupted by his departure. He also said that the head of the department, Dr R.H. Baker, was expected to take over the temporary running of the centre until its future had been decided.

David Dickson

that a Russian, Iona Andronov, who was found one day in the centre, turned out to be a reporter for the Soviet magazine *Literaturnya Gazeta* which "exposed" the centre in an article that was picked up by the world press.

There is some evidence that there were doubts in government circles in Pakistan over Nalin's centre even before the latest accusation of impropriety. Knowledgeable medical sources in India say that Pakistani scientists have been unhappy about PMRC for quite some time. It seems that Nalin's centre had been warned not to open a phial of Japanese encephalitis virus that had been brought for an experiment when it was well known that the disease does not occur in Pakistan.

Nalin admits that his centre had been engaged in work on Japanese encephalitis, but says that the work stopped some time ago. He denies that the unit ever handled genetically manipulated strains of Aedes aegypti mosquitoes as alleged by the Soviet press. Nalin said the centre's work was mainly on two species of Anopheles mosquitoes that carry malaria, a major problem in Pakistan. According to Nalin, PMRC had conducted pilot studies on control of the malarial mosquitoes by the release of sterile males and had developed an efficient way of sexing the mosquitoes to make the technique effective.

Nalin is associate professor of international health at the University of Maryland, which set up the medical centre in Lahore in 1961. Before becoming director of PMRC, Nalin worked on diarrhoeal diseases in Bangladesh at another United States funded unit run by Johns Hopkins University. That unit was expelled from India in 1975 following uproar in parliament about its activities in Calcutta. Among the reasons for its expulsion were its link with the US biological warfare laboratory in Fort Detrick and the US Navy and the fact that its American staff made frequent trips to India's border areas.

According to Nalin there is a similarity between the allegations of germ warfare that led to his expulsion from Pakistan and those raised in the Indian parliament in 1975. The Indian unit was said to be engaged in the release of genetically manipulated strains of Aedes aegypti, the vector of yellow fever which does not exist in India. The experiments to control a vector of a non-indigenous disease raised a furore and the parliamentary committee alleged that the US experiments were part of a programme to develop yellow fever as a germ warfare weapon. The Indian government closed down the New Delhi research unit despite protests from the World Health Organization (WHO) under whose aegis it was set up.

Nalin is not the first American scientist to have been expelled from the Indian subcontinent. Dr Carl Taylor, head of the Division of International Health at Johns Hopkins University, has been told by the Indian government not to come to India in any professional capacity — although he is still allowed into the country as a tourist. Taylor ran a nutrition project at Narangwal in the Punjab for some 15 years from 1960. This project, also funded by the United States, was closed down following allegations that it had been spying on the nearby major Indian airbase at Halwara. Taylor, who was recently passing through Delhi, denies any such charges.

After the closure of the WHO unit in New Delhi and the projects run by Johns Hopkins University, the Indian government has become more strict in the scrutiny of all projects involving foreign collaboration. A central agency that includes the defence adviser has been set up to give foreign-aided projects security clearance. For the past three years the United States has been trying to mount an earthquake project in Shillong and a sedimentology project in the Bay of Bengal. But both projects have been turned down — Shillong is in the politically troubled north-east region while India is sensitive about allowing geological studies in the Bay of Bengal with its huge oil K.S. Jayaraman

Space communications

UK jumps in

Britain is to have a fully-operational direct broadcasting satellite by 1986, the earliest date foreseen in a Home Office report on direct broadcasting by satellite published just under a year ago. The government's approval for the project last week reflects the urgency with which it now views the exploitation of space and information technologies. It hopes that an early start to the project will give British industry the chance to demonstrate its capabilities and to compete in the world telecommunications market.

The satellite's two direct broadcasting channels have been awarded to the British Broadcasting Corporation, largely because its plans for using them are most advanced. It was also announced last week that British Aerospace, GEC-Marconi and British Telecom are to form a joint venture called United Satellites to build the satellite system. The three companies are now consulting with N.M. Rothschild & Son, the merchant bank, over ways of raising approximately £150 million venture capital for the project.

The BBC will lease its two channels, while British Telecom may use another for telecommunications. The plan is to go straight into a fully-operational system involving two satellites in orbit (one a spare) and a third waiting on the ground. According to British Aerospace, the satellite will be based on the ECS, a telecommunications satellite built by the European Space Agency for which British Aerospace was the main contractor. Earlier suggestions that the satellite should be

based on L-sat, the second generation of European telecommunications satellites, were abandoned partly because L-sat will be unnecessarily sophisticated and too late.

The BBC will finance its operations mainly out of subscriptions for the programmes carried on one of the channels. One channel will carry repeats of programmes already shown on its two conventional TV channels and the other will be a pay-TV channel showing recently-released feature films and the pick of programmes from foreign television.

Viewers will be able to tune into the satellite by means of small domestic dishes. They may also be able to receive programmes relayed by cable from community dishes. Government plans for cable systems are to be announced shortly, although the Home Office refused this week to comment on a report in last week's *Economist* that the government is urgently considering plans to lay optical fibres between major cities and coaxial cable between households.

Nevertheless, last week's announcements are evidence of the British government's anxiety that Britain should catch up with other industrialized nations, especially France and Germany, which have been quicker to acknowledge the challenge of space and information technologies. Other moves to exploit some of the lessons Britain has already learnt in space technology, for example through its membership of the European Space Agency, may be expected within a year or so. Officials at the Department of Industry are working on a plan to increase efforts in maritime communications and on a national earth resources programme, part of which would be Britain's contribution to the European Space Agency's proposed Earth Resources Satellite.

Whether these proposals will involve Britain launching its own satellites and just how much public money will be available to fund them, however, remains to be seen.

Judy Redfearn

Information service

Online Britain

Pergamon-InfoLine, the online scientific and technical information service of the Pergamon/BPC Group, was launched this week by Kenneth Baker, Minister of State for Industry and Information Technology. Infoline will not only be available in the United States but in Britain will represent the first commercial carrier of scientific and technological databases. Although a fledgling from the home nest — its London-based computer - the company wants to break into the markets of Europe and North America and become a "world force in the growing online information industry". A new system, Video PatSearch, is to spearhead its marketing initiative.

For an annual subscription of about

£7,000 plus online connection charges (£35 per hour), Video PatSearch will provide an in-house patent search service. The system, which would sit compactly on a large desk, is the first of its kind to link an online terminal with new video disc technology to enable users to retrieve both drawings from the original patent specification and text. At present the system, which will operate in North America and Europe, is geared to the 750,000 US patents issued scine 1971.

Hitherto, a subject search of US patents on file at the US Patent Trademark Office has been possible only by using the US Patent Classification System. A search on the computer system may take only 10 to 15 minutes on the average. Video disc technology opens the way to online access to other databases covering subjects where graphics display is necessary.

InfoLine was first set up in 1978 by a consortium including the Department of Industry, the Chemical Society of London, the Institute of Electrical Engineers and the British Library. A long period of development produced only a limited number of databases and in 1980 the partners decided they were unwilling to invest further in the project. The Pergamon Group stepped in and saved InfoLine from liquidation. Several million pounds went into the setting up of an operation based on the advanced VAX 11/780 computer system.

InfoLine complements BLAISE, the online medical retrieval service of the British Library. The databases offered by InfoLine include CA Search, the Chemical Service Databases; Compendex, the engineering database; PIRA Abstracts, covering the paper, publishing, printing and packaging industries; Management Marketing Abstracts and GeoMechanics Abstracts. All of these databases have been available in the United Kingdom and Europe, via EURONET, for some time. They will now be offered to the US market. PatSearch, the database of US patents, on the US market for two years, will now be offered worldwide from the London computer.

At the opening both Kenneth Baker and Robert Maxwell, chairman of Pergamon-InfoLine, seemed optimistic that InfoLine would flourish where other government-backed schemes had failed. The minister referred to InfoLine as a "tiny bird that escaped the net" (of nationalization).

Jane Wynn

Belgian science policy

Harsh criticisms

Brussels

The parlous state of Belgium's science policy reflects the political and economic problems facing the country. The universities, which shoulder the bulk of state-financed research, are suffering particularly badly from budgetary cutbacks, and there are loud calls for the government

to make radical changes in research funding. At the same time, the unequal way in which government-funded research centres are concentrated in the more prosperous Flemish half of the country is the source of bitter complaints from the French-speaking Walloons.

The present escalation of the language war comes in response to the threat of major closures in the steel industry of the already economically depressed Wallonia, and has led Walloons to seek out other injustices to attribute to the present Flemish dominated coalition government.

Belgium has three national research centres, the Centre pour Energie Nucleaire at Mol in Flanders and the Institut de Radioéléments and the Institut National des Industries d'Extraction, both in Wallonia. Eighty-seven per cent of all the government subsidies in question are spent at Mol if the employment created by the nuclear research centre in related industries is taken into account. The last coalition government attempted to redress the balance, at least in terms of energy research, by favouring the coal industry. But this still left 70 per cent of government research funds being spent on nuclear energy compared with 6 per cent on extractive industries. Philippe Maystadt, Science Minister in both the present and previous governments, seems to have done little to soothe Walloon anger since increased expenditure on nuclear energy and solar energy mainly benefits Flanders.

The bad blood between Walloon and Flemish scientists is overshadowed by the crisis facing universities throughout Belgium. A law passed in 1971 lays down that universities are allocated a certain sum of money for each student and out of this the university has to pay for administration, teaching and research. Maystadt ruled out specialist centres for postgraduate research as an unjustifiably expensive way of funding research for a small country.

Research is thus left largely under the control of the university authorities, loosely supervised by a ministerial and an interministerial committee for science policy. In 1981, the universities spent BFr 25,000 million (£290 million) compared with BFr 15,000 (£170 million) million at other national or international research centres. This has led the universities into a trap with administrative and other running costs rising above the price indexation system and the allocations per student falling below the index increase. The universities have therefore started to cut back on research spending.

With the policy of budgetary restraint being followed by the present government, the problem is worsening. Professor André Jaumott, ex-chancellor of the Université Libre de Bruxelles, wants universities to separate their teaching and research functions, with no budget restrictions.

Maystadt has other plans. His ministry has asked Belgian industry and the state

New broom in Spain

Barcelona

Professor Federico Mayor Zaragoza, the new Spanish Minister of Education and Science, is assessing the draft of a law for scientific and technical research which should provide a framework for scientific research in Spain, something that has been almost untouched since General Franco's time.

Professor Mayor is well versed in the higher education and science politics of Spain, having occupied many key posts: rector of the University of Granada, acting president of CSIC (the Spanish science research council), president of the "Comisión Asesora" (the main fund for research grants) and "subsecretario" (vice-minister) of education and science. He is now professor of biochemistry at the Autonomous University of Madrid and director of the Institute of Molecular Biology which is a unit of the "Centro de Biología Molecular Severo Ochoa". He was elected MP for Granada in 1979, but later resigned to become deputy director-general of UNESCO.

According to the draft law, the government intends to specify who will formulate Spanish science policy, who will control the different levels of organization and how research will be financed. It puts research under the control of a secretary of state who will report directly to the prime minister. An advisory committee, mainly composed of scientists, will propose general plans and priorities and submit an annual budget for research to parliament.

No change will be introduced in the structure of research bodies such as CSIC but, to circumvent the fact that these bodies have only permanent staff, a new institution will be created, which will be a public company that will employ research staff and act as an auxiliary to research centres. This will also facilitate the exchange of scientists between research institutes and universities. The draft will be offered for discussion to research institutions before going to parliament. However, new elections will take place before March 1983 and it is unlikely that such a complex law would pass through parliament before then.

Pedro Puigdoménech

departments what their research requirements are for the coming five years. On the basis of this information, research contracts are being handed out mainly to medium-sized companies to undertake this sort of applied research. For 1980–82, BFr 1,000 million (£11 million) has been allocated on projects such as optic fibre technology for the Belgian PTT. Solvay, the Belgian chemical giant, should also benefit from funding for industry-orientated biotechnology.

Jasper Becker

Martial law in Poland

Fresh appeal

A former president of the Polish Academy of Sciences, Dr Janusz Groszkowski, has sharply criticized a group of more than 140 intellectuals and academics who last month sent to the Sejm (parliament) an appeal for the ending of martial law.

The letter, which was also addressed to the United Nations General Assembly and the UN Commission on Human Rights, said that the imposition of martial law was contrary to the right of every nation to selfdetermination, and the freedom to determine its own political status and to ensure its economic, social and cultural development. This principle, it said, was the basis of the renewal movement which sprang up in Poland after August 1980. The letter particularly deplored "the attempts to divide the nation, setting workers against soldiers and the militia, the blockade of the means of communication in the whole country, the brutal breaking of workers' strikes by the militia and army, the internment of many thousands of people in prisons and camps. Cultural life. education and learning, it said, are being "paralysed", and the media rendered

Dr Groszkowski did not disagree with the content of the letter, nor with the demand that the authorities should "put an end to this confrontation with their own people". He felt, however, that the fact that such a letter was sent to the Sejm - a body which during the Gierek regime had totally lost the confidence of the Polish nation and indeed of the Communist party - did not give legitimacy to it. The Sejm, in its present form, is a creation of the Gierek period, now being exposed as an era of distortions and corruption. To approach an institution where people linked to the Gierek era are still active, was, he felt, "a serious political error".

Dr Groszkowski's letter reveals how little the reforms of the past 18 months have affected those in high places. In September 1980, a letter from Dr Groszkowski prompted the academy to call for major reforms in Polish political and academic circles from the Sejm downwards, to clean up political patronage and restore honesty and fair dealing to public life. Any appeal from Dr Groszkowski carries considerable weight among Polish academics - in 1976 he resigned as president of the academy when he was unable to gain legal redress for Mrs Aleksandra Hankus of the Krakow Technical University. Mrs Hankus had suffered 11 years of official harassment and almost a year in prison for libel after protesting, in 1964, that her research results had been stolen by a person enjoying political patronage who then went on to gain a doctorate on the strength of her work. Vera Rich

Weapons in space

High frontiers

Washington

America should exploit its lead in technology over the Soviet Union to pursue an aggressive space-based military and industrial strategy that would provide a "technological end-run" around the Soviet military threat, according to a report published last week by the Heritage Foundation, a conservative Washington think-tank.

Describing a scheme for integrating various space-based technologies, from orbiting battleships capable of shooting down Soviet nuclear missiles to vast solar-power satellites, the report suggests that exploiting what it calls the "high frontier" of space could solve many of the nation's industrial and energy problems while providing effective military defence.

Both the political and the technical communities in Washington are sceptical of the proposal. Many question its technical feasibility; others claim that the projected cost of \$50,000 million over 10 years is unrealistically low.

However, the Heritage Foundation hopes that its proposals will capture the imagination of those in Congress and the Administration prepared to back an ambitious, space-based military strategy, and it suggests that in the long term it could prove much cheaper than developing nuclear weapons.

The general approach has some influential support. Last week, for example, Dr Richard DeLauer, Under-Secretary of Defense for Research and Engineering, told members of Congress that he believed the Soviet Union could be deploying laser weapons in space as early as 1983, and might have elaborate space battle-stations by the 1990s.

Such speculation was dismissed as "nonsense" by several members of the Defense Science Board, who claimed that an operational Soviet space-based laser was at least ten years away. However, Defense Secretary Casper Weinberger last Thursday quoted Dr DeLauer in support of the Administration's request for sharply increased defence expenditures to offset the Soviet lead in areas such as laser weaponry. (President Reagan is asking for \$433 million for research into laser weapons for the fiscal year 1983, compared with about \$340 million this year, and only \$165 million two years ago).

The Heritage report suggests a three-layered defence against a Soviet missile attack. The first would consist of 432 satellites orbiting the Earth, each carrying conventional heat-seeking rockets, and capable of detecting and destroying a Soviet missile soon after launch. This would be backed up by satellites with more advanced weaponry capable of intercepting re-entry vehicles in mid-course. Finally US missile silos would be protected

by non-nuclear projectiles capable of blowing up incoming warheads.

Heritage officials claim this scheme would both defend the United States against Soviet missiles with a 95 per cent chance of success, and could protect Western Europe against intermediaterange missiles. Their report encourages the development of space-based industrial processing and of the solar power satellite, enthusiastically endorsed by many leading aerospace companies but reported on sceptically last year by both the National Academy of Sciences and Congress's Office of Technology Assessment.

Assessment of the technical feasibility of the proposed programme varies widely. There is general support for some of the less radical technology that would be involved, such as the missiles used for silo protection, but greater doubt about the advanced systems needed to detect and destroy Soviet missiles.

Other uncertainties surround management, cost and political acceptability. On the first, the foundation suggests a dedicated national effort comparable with the Manhattan Project which, it claims, could have an operational system in place within five or six years. On cost, it estimates a total of \$50,000 million, of which \$35 million would be redirected from other areas of the defence and intelligence budgets, and the remainder from the National Aeronautics and Space Administration. General Daniel Graham, who was military adviser to Mr Reagan during the 1980 election campaign and is a former director of the Defense Intelligence Agency, headed the nine-member panel which put together the report. He admitted that this figure might be unrealistically low, but added that "even if we are 100 per cent under, we are offering a strategic bargain".

The whole proposal, however, raises arms control problems that may mean indefinite delay. For example, it would fuel Soviet criticism that the space shuttle is essentially a military programme. The satellites might also be accused of violating the UN Space Treaty, signed by both the United States and the Soviet Union in 1967, which prohibits the stationing of weapons of mass destruction in space.

Finally any hint of a shift in military and strategic planning away from the MAD doctrine would resurrect the heated technical debates of the late 1960s over the adequacy of defence measures, at that time over anti-ballistic missiles. Critics such as Dr Kosta Tsipis of the Massachusetts Institute of Technology and Dr Richard Garwin of IBM have been quick to point out that any space-based weapons system based on sophisticated communications is highly vulnerable to enemy attack.

General Graham said that a copy of the report had been passed to the Office of Science and Technology Policy, which is preparing a report on the future of the American space effort, and whose director, Dr Jay Keyworth, has been keen to explore ways of exploiting the capability of the space shuttle. Dr Keyworth, however, is not expected to show much sympathy for the more radical technical proposals being suggested by the Heritage Foundation. He has already resisted congressional pressure to build and operate high-energy laser battle-stations for space defence against ballistic missiles. Orbiting battleships are unlikely to gain more approval — although they may get on to the list of advanced military technologies which is expected to appear on the Agenda of the new Science Council. David Dickson

Bangladesh conference

Fertile minds

Dacca

Agricultural and rural development were dominant themes at the conference of the Bangladesh Association for the Advancement of Science held in February at Joydebpur, twenty miles north of Dacca. The conference was opened by Justice Abdus Sattar, President of Bangladesh, who urged scientists to bend their energies towards self-sufficiency in food, population control and the development and exploitation of indigenous resources.

Professor A. K. Aminul Huq, this year's president of the association, pleaded in his address for the planned development of manpower to meet the needs of the domestic agricultural industry and of those "manpower importing countries" in which people from Bangladesh find jobs. He said more use should be made of agricultural wastes as a source of energy and pleaded for more government support for science and technology, given the enormity of the problems with which these professions can assist.

More immediate assistance with agricultural development is likely to flow from an agreement now reached between the government and the Saudi Fund for Development. The fund will provide US\$80 million — part grant, part low interest loan — towards the cost of the Chittagong fertilizer factory, which should be completed in 1985.

This project will cost \$467 million, and the plant will have a daily output of 1,000 tons of ammonia fertilizer and 1,700 tons of urea fertilizer. It has been financed by the Asian Development Bank, the Overseas Economic Corporation of Japan, the Abu Dhabi Fund for Arab Economic Development, the Canadian International Development Agency and the Islamic Development Bank.

When signing the most recent loan agreement, the managing director of the Saudi Fund for Development, Mr Mohammed Abdullah Al-Sugair, said that he would be recommending that the fund should be more sympathetic to the needs of Bangladesh. The fund is supporting six projects at a total cost of 727 million Saudi rials (£116 million).

M. Kabir

CORRESPONDENC

Flower power

Sir — It is to be hoped that the outcome of Indian production of paper from water hyacinths, Eichornia crassipes (Mert.) Solms (E. speciosa Kunth.), reported by Jayaraman1, will be more successful economically than previous attempts to use the plant's fibre in this way.

His statement that "all efforts so far have been directed at eradication of the weed" overlooks some interesting developments elsewhere. It is only in slow-moving waterways that the species becomes a pest. Soon after its introduction into Hong Kong and South China, in the first decade of this century2. came cultivation, primarily as a feed for pigs. taking full advantage of the rapid multiplication of the plant. It is grown in South China today, in ponds and flooded fields, as a feed for livestock and poultry3. Burnt, it can be a useful manure, because of its high potash content.

Where it is plentiful, and not required for other purposes, straw is probably preferable as a source of paper, because of its higher yield4.

WILLIAM GARDENER

Colchester, Essex, UK

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Wages of war

SIR — It would be a disservice to the scientific community if the assertions of R.G.S. Bidwell (Nature 11 February, p.452) were allowed to stand unrepudiated. This author clearly sees a direct link between the imposition of sanctions and the waging of war, and appears to welcome the opportunity of contributing to it. He refers disparagingly to the activities of pacifists in the Great Wars, not realizing that their efforts in the First World Disarmament Conference were within a hair's breadth of creating conditions which would make war an impossibility. Sanctions, on the other hand, can do nothing but increase the likelihood of war, because they only serve to nourish suspicions of malevolent intent on both sides. Never have Soviet scientists that I have spoken to expressed any desire to destroy us, as Bidwell is close to suggesting.

No scientist working for the benefit of humanity can surely wish to be associated with the notion that science is "ours", not "theirs", because we have paid for it. If the fruits of science are not made available to others, then it is of no earthly use. If the science we do in our laboratories is considered unsuitable for widespread application, it is better left undone. What we pay for is the prestige of making a discovery, the benefit to our industry of detailed technical knowledge and experience, and the chance to contribute to mutual understanding between nations, but not for the right to deny this knowledge to the rest of the scientific community.

Lack of understanding through insufficient information gives rise to some of the grossest iniquities in the world today. The eclipse by the Polish situation of the tragic events taking place in El Salvador is a direct result of restriction of flow of information. It is

important that scientists should avoid involvement in such a practice, and refrain from endorsing the dubious and hypocritical moral judgements of our cold-war strategists.

ROGER R.C. NEW

Department of Biochemistry, University of Liverpool, UK

Myth or fact?

Sir - Is the creation versus evolution argument really irreconcilable?

To those who cling to every word of the Bible nothing can be said. But surely the Old Testament and other ancient texts are the repository of ancient knowledge. Perhaps the creation is a distant "folk memory" reinterpreted by succeeding generations - of events that actually took place at a time (15,000 vr BP?) beyond documented recall.

The geological evidence is that marked changes in the environment took place at the Pleistocene/Holocene boundary. Could the ancient accounts of the "beginning of the world" be telling us that the changes at this time were more profound than the geologists normally consider? Looking at ancient legends about the beginning of the world it is striking how the same motifs occur time and again: darkness over the face of the oceans; little or no dry land; the low heavens (the firmament) yet to be separated from the earth; a creator or creators usually of remarkably human kind.

Mythologies come down to us in garbled form; and modern science knows less than it cares to admit. So the split on this issue is difficult to understand.

There is a geological theory with a great deal of evidence in its favour that, in outline at least, can explain the drastic world changes alluded to in ancient legend. But for some reason it has little or no currency: for this reason one hesitates to elaborate

R.B. TOPHAM

Abingdon, Oxon, UK

SIR — It seems to me that creationist science is at least as good as the evolutionary theology propagated by Jon Marks (Nature 28 January, p.276). To take his points in order: the Hebrew used in Leviticus 11: 19 and translated "bird" clearly denotes all warm blooded flying animals since the distinction between birds and mammals was unknown when it was written: Jesus did not ascend to paradise (or heaven) 3 days later as Marks claims. He ascended to heaven bodily at Pentecost 40 days later; what Jesus meant in Luke 23:43 was that both the thief and He would that day be in heaven in their spirits or souls. What Origen and Maimonides thought about the Bible is not pertinent; they were human and could err. There is no doubt, however, that Jesus Christ believed in a 6-day creation, a literal Adam, a real Garden of Eden, an actual world-wide flood and a real whale that swallowed and regurgitated Jonah.

As for the morality of the Bible, neither the rape of Dinah nor the seduction of Lot by his daughters is held up as a good thing. Rather they are consequences of the fall of Man, just as Dr Marks' cynical unbelief is. The execution of Sisera was surely justified for war crimes; he had oppressed Israel harshly for 20 years (Judges 4:3). We do not know all the details but no doubt there was something particularly appropriate that he should be killed by a woman with a tent peg. If Dr

Marks read his Bible with understanding he would realize that Ecclesiastes 3:9 is a perfect description of the futility of man without God, and one that fits Dr Marks himself.

It may be that someone who knows nothing but the 750,000 species of insects is a colossal bore, but someone who knows that, understands every language known to man, comprehends the whole of mathematics, physics and even anthropology would probably consider Dr Marks and his petulant fist waving at his Creator a little boring.

That chimpanzees and humans should be so similar genetically merely shows their Designer recognized a good thing when He saw it.

For Dr Marks to accuse creationists of fraud is a little thick. For whose benefit was the Piltdown fraud perpetrated? What about Haeckel's faked photographs showing that embryology recapitulates phylogeny?

As for obscurantism; the word means the denying of inquiry. Is it not the evolutionists who hold that the theory of evolution is unchallengeable and must be accepted as fact?

T.J. HAMBLIN

Royal Victoria Hospital, Bournemouth, UK

Culture conscious

Sir — In a recent issue (*Nature* **294**, 42; 1981) Adrienne Zihlman reviewed Sarah Hrdy's The Woman That Never Evolved and my own The Evolution of Love.

Professor Zihlman concluded that "Both of these books join the growing stacks of sociobiological attempts to integrate genes and human behaviour. They fail by ignoring the intervening levels. . ." and that "the book that has not yet been written is one that. . . integrates culture and biology". In fact my second chapter contains an elaboration of the interaction of cultural and biological evolution, and my view that cultural evolution has probably "initiated or promoted in humans a far greater number of new behavioural trends than biological evolution has". My index shows that cultural evolution is discussed or mentioned significantly on at least 46 pages of the total of 291 pages of text. SYDNEY L. W. MELLEN

Vevey, Switzerland

SIR - In her review of my book The Woman That Never Evolved, Adrienne Zihlman quotes me out of context and implies that I state women's sexual activity is "assertive and temporarily insatiable". But I was referring to a monkey in oestrus, and was contrasting such monkeys with human females. I wrote: ' what earthly relevance does the conduct of this monkey have for understanding her culturebearing cousin, whose solicitations are sedate. self-conscious, often elaborate in their subtlety and indirection?" (p.160).

My point was to show that cultural practices such as purdah and clitoridectomy, institutions such as marriage, and perhaps especially the myths and values that are a very real component of each human individual, have profound effects on the sexual behaviour of women (pp.179-187).

SARAH BLAFFER HRDY

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NEWS AND VIEWS

Creation, evolution and high school texts

from Robert M. May

ANYONE who has seen Clarence Darrow—as played by Spencer Tracy—triumph over William Jennings Bryan in the powerful movie *Inherit The Wind* will be pardoned for thinking that the Scopes Trial helped establish evolution over creation in the United States in the 1920s. This impression is, indeed, fairly widely held, and is explicitly affirmed in at least one university-level biology text.

To the contrary, Grabiner and Miller¹ have shown that the pressures exerted by fundamentalists in the 1920s were successful in bringing about the systematic removal of references to evolution and Darwin in high school biology texts, particularly in the Southern States. Before the Scopes Trial in 1925, several of the widely used texts discussed evolution1: Hunter's A Civic Biology (American Book, 1914), from which Scopes himself taught, had a three page section on evolution together with other associated material elsewhere in the book; Gruenberg's Elementary Biology (Ginn, 1924) was outspokenly evolutionist, with several chapters devoted to the subject and classification discussed from an evolutionary standpoint; Moon's Biology for Beginners (Holt, 1921) had several chapters on evolution and a picture of Darwin as the frontispiece. Other successful texts were more reticent: Smallwood, Reveley and Bailey's New Biology (Allyn and Bacon, 1924), possibly the most widely adopted text of the time, gave about two pages to evolution and omitted any discussion of the origins of man; Clement's Living Things (Iroquois, 1924) gave evolution only brief mention; and Peabody and Hunt's Biology and Human Welfare (Macmillan, 1924) explicitly excluded evolution. Grabiner and Miller give a simple rule for identifying texts published in the decade following 1925: "Merely look up the word 'evolution' in the index or the glossary; you almost certainly will not find it". Concerned about the effect the Scopes Trial might have on sales of his text, Hunter rechristened the 1926 edition New Civic Biology and removed most of the explicit discussion of evolutionary ideas; the word 'evolution' is no longer in the index. The 1926 edition of Moon's Biology for Beginners has the frontispiece portrait of Darwin replaced by a cartoon diagram of the digestive system, although the substantial treatment of evolution in the

text is retained and 'evolution' remains in the index (at last disappearing, however, in the 1933 edition). The treatment of evolution in Smallwood et al.'s New General Biology (1929) is even more perfunctory than in their earlier New Biology, and again the entry for 'evolution' is removed from the index. The most widely used text in the 1930s was Baker and Mills' Dynamic Biology (Rand McNally, 1933), which discusses evolutionary ideas in the final chapter; the book², however, manages to avoid ever using the term 'evolution' and concludes with the extraordinary statement that "Darwin's theory, like that of Lamarck, is no longer generally accepted'

As emphasized by Simpson³, it is not so much that the Scopes Trial precipitated these changes, but rather that both the trial and the changes were consequences of the upsurge of anti-intellectual conservatism and biblical literalism during the period in question. The essential point, however, is that evolutionary biologists were deluded in seeing the drama enacted in the Tennesee courthouse as a significant victory. The real battles were being fought over state and local decisions about which textbooks to adopt for high school biology courses, and here creationists advanced on a broad front throughout the 1920s and 1930s.

We would do well to keep these facts in mind today. Even as the community of professional biologists takes comfort from Judge Overton's incisive and unequivocal verdict against the state of Arkansas' law mandating equal time for 'creation science' in the biology classroom, there are signs that new editions of major high school biology texts are being eviscerated.

As stressed by Nelkin⁴, a sociologist whose Science Textbook Controversies and the Politics of Equal Time (MIT Press, 1977) is the canonical book on this general subject, it is "too early to get a detailed reading on actual changes; they are just being implemented and publishers won't talk". But there are some clear signs, summarized recently by Zuidema⁵. The index to the 1973 edition of Biology: Living Systems (Charles Merrill) gave 17 lines of page references under the heading

'evolution'; in the 1979 edition, 'evolution' is indexed in 3 lines. Recent editions of three Harcourt Brace series texts omit all mention of Charles Darwin, and one excludes the index entry 'evolution' (though the subject is covered in the text). This is sad, as Simpson³ has praised Harcourt Brace for 'consistently and effectively oppos[ing] anti-evolutionist pressure' in the earlier period around 1930–1960. Another brief survey⁶ shows the 1977 edition of an Otto and Towle high school biology text has diminished the coverage of evolution by one-third compared with the 1973 edition.

Taking a different tack, some texts now include material on the Genesis account of creation, or on creation myths generally^{5,6}. Books in which special creation now appears include two editions of Biology: An Inquiry Into the Nature of Life published by Allyn and Bacon in 1974 and 1977, a 1974 Smallwood and Green text, and the 1980 Houghton Mifflin text Biology: The Science of Life. These books can be characterized as teaching about creationism, but not supporting it. More controversial is Biology: A Search for Order in Complexity (Zondervan), written by Moore who is a 'born again' professor of natural science at Michigan State University and a founder of the Creation Research Society. The book was chosen as one of seven officially approved biology texts by the Indiana State textbook commission in 1975, and in two of Indiana's districts was the only ninth-grade biology text available to students. Later, in 1977, the book was barred from use in public schools in Indiana as sectarian; it was relegated to library use as a reference work in Texas, but remains approved by state commissions in Alabama, Georgia, Oklahoma and Oregon5.

Underlying much of this is the system whereby 19 of the 50 United States have prescribed selection systems under which textbooks are approved for adoption in schools. According to rules which vary from state to state among these 'adoption states', one or several books may be approved for school use at a given level?. These texts may then be provided to schools, paid for with tax dollars; alternative books may be used but they must be bought by the individual schools or by individual students. These 19 'adoption states' are preponderantly southern, but importantly include

Robert M. May is Class of 1877 Professor of Zoology at Princeton University, New Jersey 08544. California (which accounts for about 10 per cent of the national textbook market) and Texas (which currently has an annual budget of \$45 million for high school textbooks)7. Given the highly competitive nature of the textbook market, the pressures that this system — with all its political and populist overtones — puts on publishers is obvious. Typical, and understandable, is the havering of one publisher about his company's text8: 'evolution runs like a thread throughout, but is mentioned specifically only in the last chapter".

Why are creationists not a force in Europe? The reasons are many and varied, but one simple set of numbers helps to characterize the difference. Writing of "revived dogma and new cult", Martin9 observes that in Northern Europe, around three to five per cent of those nominally Protestant are found in church on any given Sunday. In contrast, the latest Gallup polls show 51 per cent of all American teenagers in church on any given Sunday and at least one-third of these are fundamentalists.

Grabiner and Miller's conclusions1

about the Scopes Trial era have a message for today. "Readers may choose their own villain in the story we have told. Like us. some will find the greatest culpability in the scientific community itself, for the largescale failure to pay attention to the teaching of science in the high schools. Others will blame the textbook authors and publishers for pursuing sales rather than quality. Some will attach blame to the politicians who exploited anti-evolution sentiment to get into, or remain, in office . . . But whatever the lesson one wishes to draw from the history of biology textbooks since the Scopes trial, we think the story itself is worth knowing. That the textbooks could have downgraded their treatment of evolution with almost nobody noticing is the greatest tragedy of all."

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VELOCITY OF LIGHT TO BE CONSTANT — OFFICIAL

from Bridget Marx

ANYONE wanting to measure the speed of light had better do it soon. Indeed, it may already be too late. From the autumn of 1983 no one will be able to follow the example set by such illustrious people as Fizeau, Foucault, Maxwell and Michelson. Why is this? If the proposed new definition of the metre is put forward to and accepted by the General Conference on Weights and Measures, then it will entail the introduction of a fixed numerical value, 299,722,458 m s⁻¹, for the speed of light. Then 'c' will no longer be a measurable quantity but will be a fixed number.

The present situation is as follows. We have the second, the unit of time, defined in terms of a transition frequency near 9 GHz from a caesium atom: the second is the duration of 9,192,631,700 periods of this radiation. We have the metre, the unit of length, defined in terms of a transition wavelength at 605.8 nm from a krypton atom: the metre is 1,650,763.73 times the vacuum wavelength of this radiation.

Using these two defined units, we can measure the speed of light, in metres per second, in one of several ways. The earlier investigators used the technique of actually timing a pulse of light travelling over a measured distance. But light travels so fast that this is a difficult technique to use at high precision. More recently, accurate measurements of c have been made by

using the relationship between frequency, ν , and wavelength, λ , which holds true for all electromagnetic radiation: $v = c/\lambda$.

An accurate measurement of ν , in inverse seconds, and λ , in metres, results in an accurate experimental value of c. One difficulty is that the second and the metre are defined in terms of radiations at very different parts of the electromagnetic spectrum. These measurements therefore involve 'frequency-chains' from the 9 GHz Cs frequency to the 4.95 × 105 GHz Kr frequency - a series of oscillators the outputs of which form bridges to span the spectrum, so that frequencies in the optical region can be compared with those in the radiofrequency region. So for one particular radiation source, usually a laser, the frequency and wavelength can be found by comparison with those quantities which define the units of time and length, giving a precise experimental value of c.

What limits the precision of such an experiment? It is partly determined by how well you can do it, but also by the limiting accuracy with which the basic units can be realized. That is, it depends on how well Cs clocks and Kr lamps can be reproduced internationally, and their frequency and

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wavelength respectively measured. The realization of the second through the Cs clock has a reproducibility of better than 1 part in 1013. Despite the fact that wavelength comparisons can be made at an accuracy of parts in 1011, the realization of the metre through the Kr lamp, which has a relatively broad spectral profile, is reproducible only to about 4 parts in 10°. As saturated absorption-stabilized lasers operating in the visible are internationally reproducible to a few parts in 1011, there is obviously scope for an improved definition of the metre.

From what has been said, it might seem that a good redefinition of the metre would be to specify it, as at present, in terms of a wavelength, but to choose as this wavelength the most accurate that can be generated with a visible laser source. (For practical reasons it is easiest to use visible radiation for length measurement.) This would indeed be possible, but it would not be easy to decide which laser would be best for the definition. It would also mean that if a better, more accurate laser became available in a few years, then the definition might need to be changed again. It has therefore been decided to use the high precision of the frequency standard and to define the metre in terms of the second and a fixed value for the speed of light. The redefinition most likely to be recommended is that "the metre is the length equal to the distance travelled in a time interval of 1/299,792,458 of a second by plane electromagnetic waves in a vacuum" (Comité Consultatif pour la Definition du Metre BIPM, 6th session, Sèvres; 1979). In this definition, c becomes simply a conversion factor between time and length.

This is a concept that we are familiar with through the use of the 'light year' as a unit of length. Both the proposed definition and the light year assume that the speed of light is constant but, as has been pointed out earlier (W. R. C. Rowley, Phys. Bull. 31, 334;1980), the constancy of c is also assumed implicitly in the present definition of the metre. What is fixed in the krypton atom is its energy level structure, and so the transition energy E. The wavelength emitted from the transition is given by $\lambda = hc/E$, where h is Planck's constant. The value of c to be used in the new definition has been chosen to be that measured with the existing metre, so that continuity is maintained.

The proposed redefinition of the metre will, however, rely heavily on laser sources for its realization. Any radiation of known frequency will automatically be of known wavelength, because c is fixed, and so may be used as a wavelength and length standard. And as the reproducibility of the frequency standard is improved, so the accuracy of the metre will follow it which is good news for everybody, except for those who have always wanted to measure the speed of light but haven't got round to it yet.

Gravity waves seeding ionospheric irregularities

from Jürgen Röttger

THE upper atmosphere of the Earth, particularly above 50 km altitude, is partly ionized by solar radiation. The electron densities that result display diurnal, seasonal and other quasi-regular variations, the peak densities occurring within the so-called F-region of the ionosphere (see Fig. 1). Superimposed on these comparatively predictable features of ionospheric behaviour are disturbances such as those caused by solar flares and magnetospheric storms. Other irregularities, detectable by means of radio waves reflected or scattered from the ionosphere and unrelated to any obvious extraterrestrial disturbance, are often difficult to explain. However, recent work has demonstrated that the neutral atmosphere plays a crucial role in their generation.

Their usual name apart, irregularities in the ionospheric plasma are rather common and regular features. Particularly near the equator, F-region irregularities occur quite frequently during the night and often exhibit a rather ordered, even periodical occurrence1,2. Various plasma instabilities are responsible for generating irregularities with dimensions less than a few kilometres3 while dynamic processes in the neutral atmosphere are responsible for larger-scale periodical features of equatorial F-region irregularities1-5. These processes are internal gravity waves which cause travelling ionospheric disturbances (TIDs) in the ionized part of the upper atmosphere. Recent research has shown that the disturbances can be amplified in the equatorial F-region due to mutual coupling with dynamic processes in the ionospheric plasma6. The resulting phenomena are periodic modulations, wave breaking as well as seeding or triggering plume-like irregularities.

Understanding of the equatorial F-region plasma irregularities has advanced in the present decade by the combination of theory and numerical simulations with data from ionosondes, backscatter radars, rockets and satellites and from analyses of scintillations of satellite signals1-3. The irregularities, which are also called 'equatorial spread-F', commence after the bottomside profile of the post-sunset F-layer has steepened. Steep vertical gradients of plasma density can be unstable to the classic Rayleigh-Taylor instability because a heavy fluid the plasma - is supported against gravity by the Earth's magnetic field. Perturbations in the plasma density of the bottomside F-region develop into density depletions and small-scale irregularities. Irregularities at the bottomside of the ionosphere occur in wave trains of patches

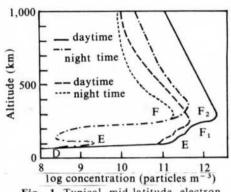


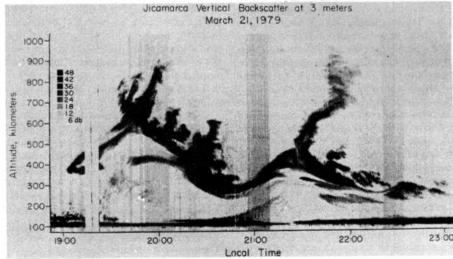
Fig. 1 Typical mid-latitude electron concentration profiles in the ionosphere. The larger daytime and night-time values occur at sunspot maximum, the smaller at sunspot minimum.

separated by some 100 km (ref. 4) and move eastwards and downwards. Plume-like irregularities are caused by the depletions rising, like bubbles in a fluid, to the topside of the F-region^{7,8}.

The new evidence⁶ that gravity waves initiate equatorial spread-F irregularities comes from observations carried out with the Jicamarca radar located close to the magnetic equator near Lima, Peru. The radar transmits at 50 MHz with 1 MW pulse power from a phased-array 300 m × 300 m antenna pointing nearly vertically. Backscattering from field-aligned spread-F irregularities of scale sizes of one-half the radar wavelength can be observed and radar pictures of the life history of spread-F irregularities recorded7,9,10. One spectacular event recorded by Kelley et al.6 is shown in Fig. 2. The irregularities first occurred after sunset at the bottomside of the F-layer and, during the evening, the bottomside irregularities underwent nearly two full cycles of a quasi-periodic vertical motion. In both cycles, distinct plume-like structures extended to the topside of the F-laver and during downward motion, plumes were generated at intervals of about 20 min. These plumes are the signatures of plasma bubbles rising through the ionosphere up to altitudes of about 1,000 km. Because of their alignment to the Earth's magnetic field, the bubbles extend in stretched tubes to the north and south of the magnetic equator.

Since magnetospheric influences on the periodic bottomside modulation of the irregularity pattern can be ruled out, Kelley et al.6 argued that gravity waves have a role in this process in agreement with earlier investigations relating internal gravity waves to equatorial spread-F4,5,11,12. This relation is rather intriguing since it allows a spatial resonance mechanism, as proposed by Whitehead13, to play a part - most effectively in the post-sunset equatorial F-region where the ionospheric plasma, drifting downwards at some 10 m s-1, has similar downward phase velocities to those of gravity waves. An eastward plasma drift of 100-200 m s-1 is also observed14,15, matching typical horizontal phase velocities of gravity waves. When this matching occurs, the ionization pattern due to the wave stays in the same position relative to a drifting ionization parcel, and the wave-like perturbation in the ionization consequently increases. Even very small disturbances can be strongly amplified by this resonance and can reach the non-linear regime¹². Steep ionization gradients and depletions appear as a consequence. These were assumed to be the periodic source regions, that is, the primary perturbations necessary to initiate the Rayleigh-Taylor instability at the bottomside F-layer4,12. As observed with radars, the generated ionization depletions or bubbles then drift upwards to the peak and the topside of the ionosphere^{7,8}. Independent of the radar observations, deep depletions (bite-outs with only some per cent density of the surrounding plasma) were detected by in situ measurements with rockets and

Fig. 2 Radar map showing echo traces due to backscatter from equatorial spread-F irregularities at altitudes larger than 200 km.



Jürgen Röttger is at the Max-Planck-Institut für Aeronomie, 3411 Katlenburg-Lindau, FRG. satellites2,16. The periodic features of spread-F plumes or bubbles also cause intense scintillation of satellite signals¹⁷ and adversely affect radio communication links.

Including the Doppler effect due to neutral background winds, Kelley et al.6 discussed the range of gravity waves which could match their data. They found that the long-period huge ionization perturbation observed in Fig. 2 would require a vertical velocity amplitude of 100 m s⁻¹ much too large to be realistic. They concluded that a gravity wave itself cannot explain the magnitude of disturbance but seeds the initial perturbations even if spatial resonance is only partially attained. Provided an initial finite amplitude perturbation exists due to gravity waves, they assumed that a nonlinear Rayleigh-Taylor growth for long-wavelength structures can be sufficiently high to explain the long-period modulation in Fig. 2. Smaller disturbances, with shorter periods of about 20 min, occurred during the downward motion and may be due to shorter-period gravity waves superimposed on the long-period perturbation.

These recent results establish that the life history of equatorial spread-F irregularities is a multistep process, starting with initial large-scale perturbations due to gravity waves which seed the generation by plasma instabilities of a cascade of smaller-scale

irregularities. As shown for the equatorial region, processes in the ionosphere cannot solely be treated from the viewpoint of plasma physics, but neutral atmosphere processes, namely internal gravity waves. have to be regarded as well. Since these waves presumably are generated in the troposphere^{6,18}, intriguing research on the coupling between the lower and upper atmosphere remains for the future.

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Molecular basis of growth hormone deficiency

from Mike Wallis

THE first use of recombinant DNA technology to investigate the molecular basis of defective production of pituitary growth hormone (GH), one of the causes of dwarfism in man, is described in a recent paper by Phillips, Hjelle, Seeburg and Zachmann¹. Three related Swiss families were studied. The growth hormone deficiency is inherited2 in an autosomal recessive mode; the six parents had normal stature but the growth of four of nine children was stunted. Using a cloned cDNA probe for human growth hormone (hGH), Phillips et al. showed that each of the four stunted individuals was homozygous for a deletion of at least 7.5 kilobases of DNA which includes most, or perhaps all, of the normal human growth hormone gene sequence. The dwarf individuals show a complete lack of GH (but not of other pituitary hormones) and when treated by the administration of hGH develop antibodies which neutralize its effect.

Phillips et al. took high molecular weight DNA prepared from blood samples from the dwarfs, other members of the same families and from control (unrelated) indi-

viduals and digested it with a variety of restriction endonucleases. The DNA digests were fractionated by electrophoresis, transferred to nitrocellulose filters (Southern blotting) and hybridized with a labelled hGH cDNA probe. For each restriction enzyme digest, several hybridizing bands were detected corresponding to various members of the family of genes known to be related to cDNA for hGH3. In each case, the affected (GH-deficient) individuals lacked one of the hybridizing bands, and their parents and most (but not all) of their siblings possessed less of this component (as would be expected for heterozygotes) than did normal individuals.

Interpretation of the data is complicated by the presence of at least two types of hGH gene (hGH-N and hGH-V; normal and variant) and probably at least three types of gene for the closely related hormone placental lactogen. The DNA sequence(s) coding for the placental lactogen are suffi-

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ciently similar to those for hGH for extensive cross-hybridization to occur. Phillips et al. detected DNA fragments corresponding to at least the two hGH genes (hGH-N and hGH-V) and one placental lactogen gene, and their observations were apparently complicated further by the existence of an allelic polymorphism occurring in a placental lactogen gene. Their work suggests that the hGH-N and hGH-V genes are non-allelic, and that the hGH-V gene (which could produce a growth hormone differing from normal hGH at about 14 residues) is either nonfunctional or produces a poorly functional product in the GH-deficient individuals, all of which retain this gene. It is still possible, however, that the hGH-V gene is expressed and functional in normal individuals: deletion of DNA associated with the hGH-N gene in the dwarfs might lead to reduced expression of the hGH-V gene.

The complexity of the organization of the human GH and placental lactogen gene family contrasts with the situation in nonprimate mammals. In the rat, for example, cDNA to rat GH cross-hybridizes with only a single growth hormone gene, the structure of which has been elucidated in detail⁴⁻⁶. The gene contains four introns. The related protein hormone prolactin is also represented by a single gene in the rat genome^{4,7}; again, the gene contains four introns, the positions of which correspond to those in growth hormone, but the sizes of which are far greater (on the average by a factor of five). It seems likely that the genes of the hGH-human placental lactogen family underwent a period of rapid evolution, including gene duplication and multiple substitutions, during the evolution of the primates; not only does the organization of the genes for GH-like proteins in primates differ markedly from that in non-primates, but the amino acid sequences of hGH and non-primate GHs are also very different8.

The kind of approach used by Phillips et al.2 should be applicable to the investigation of other types of hGH deficiency. Much human dwarfism, however, does not result from simple lack of GH. Other causes of short stature are recognized, but many children are very short despite normal immunoreactive GH levels and the absence of any other obvious cause. Recently, Rudman et al.9,10 have proposed that a substantial proportion of such 'normal-variant short stature' (NVSS) children are short because they have a defective GH, which is still detectable by radioimmunoassay. Growth may be inducible in such children by exogenous GH. This work has aroused great interest in the medical journals, though some aspects of it remain controversial¹¹⁻¹³. Application of recombinant DNA techniques should help elucidate the nature of such GH defects.

Dwarfism caused by lack of GH can often be treated by injection of hGH (but not non-primate GH) and such treatment

has been applied effectively in the UK and many other countries using GH extracted from human pituitary glands. The supply has barely met the demand, however, and recently increasing demand and falling supplies have aggravated the situation (see Nature News 294, 200; 1981). Application of recombinant DNA technology has led to the production of hGH from bacteria^{14,15}, and clinical trials with such material are starting. If the prevalence of GH-treatable NVSS children is as high as Rudman and his colleagues suggest (perhaps two to ten times as many as those now treated with GH), the need for hGH produced by genetic engineering will be considerable. But the hGH produced in bacteria is unlikely to be completely without problems. The material currently being tested has a structure slightly different (due to an additional N-terminal methionine residue) from the pituitary-derived hormone, and there is no guarantee that bacterially derived material will be substantially less expensive than that obtained from pituitaries. (Complete treatment of a GH-deficient child currently costs about £10,000.)

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St Patrick and the bacteria

from J.S. Jones

THERE are two models of the absence of snakes from Ireland, neither of which, as the Creationists delight in reminding us, is directly testable. The older of these has it that St Patrick banished into the sea all manner of noxious and creeping things from his adopted land. More recent theories - such as MacArthur and Wilson's equilibrium theory of insular biogeography¹ — see this absence as a result of the unique faunal history of islands, which is profoundly influenced by their size and isolation.

The classic studies in island biogeography have been of birds but, surprisingly, more direct opportunities to test McArthur and Wilson's theory are now coming from investigations of the patterns of bacterial and viral infections in man. Not only does it appear that the spread of disease in isolated islands of human population accords well with the theories of biogeographers, but even changes in the bacterial flora of a single individual and an 'archipelago' of his family, friends and pets follows the same ecological rules as those thought to control the biology of island birds.

The equilibrium theory of island biogeography

attempts to predict the number of species on an island from a balance between immigration and extinction. Immigration depends largely on the island's distance from a mainland source. The rate of extinction is related to the island's area; the smaller the island, the fewer the members

The MacArthur and Wilson theory

of a particular species which it can support and the greater the chance that the species will become extinct through random fluctuations in population size. Considerable turnover will thus be expected; although the number of species on an island will be constant, their identity will change with time, with the accidents of immigration and extinction leading to a dynamic equilibrium.

In an important new book, Williamson² takes a critical look at some of the many attempts which have been made to interpret the biology of islands in terms of the equilibrium theory. He points out that although it has been known for more than a century that there is a relationship between the number of species and an island's area and isolation, the evidence for a constant random turnover on islands is much less convincing. A forty year study of the birds of Skokholm, for example, shows that there has been almost no species turnover during this period. Other claims3 that the population history of island birds in the Pacific and elsewhere supports the McArthur-Wilson view of species turnover on very inadequate are based information4.

Can more adequate data be taken from the huge body of information on the ecology of infectious disease? If infection is regarded as the immigration of a disease and cure as it's extinction then it seems that it may.

Humans on islands

Measles occurs only in man and, because immunity follows infection, the virus can persist only in a large population where there is a continuous supply of susceptible persons⁵. A study of 19 island populations⁶ shows that measles periodically dies out in

all those containing less than 500,000 people, and that (exactly as claimed by MacArthur and Wilson) the probability of extinction of the virus is greater on small islands than on large. The rate of immigration also has a major effect on persistence and turnover; in Iceland (where records of the incidence of measles date from 1869), there were intervals of up to seven years between epidemics before 1938, a period during which there was little movement of infected individuals into the island. After 1945, when immigration was common, the time between epidemics was never more than two years7.

A similar pattern of turn over has been traced in an isolated Eskimo village from the persistence of specific antibodies in those who survived polio infection8. One strain passed through the community 25 years before the investigation; another 15 years before, and a third only within the previous year.

Black9 points out that most primitive human communities exist as islands of population, and that many of their diseases show such patterns of turnover, with a balance between the immigration and extinction of pathogens which is related to each island's size and isolation. In Amazonian tribes, for example, the incidence of particular infectious diseases differs greatly (and apparently at random) between the various isolated communities. In different villages the proportion of the population with antibodies to measles varies from 0 to 98 per cent, to rubella from 0 to 95 per cent and to parainfluenza B from 0 to 89 per cent. Disease incidence again results from a dynamic equilibrium between a chance immigration and a period of persistence followed by extinction.

Humans as islands

To bacteria, all men are islands, safe refuges in an inhospitable sea. The human intestine is such a favourable habitat that every healthy individual has ten times as many symbiotic bacterial cells as he has cells of his own11. Gel electrophoresis of a sample of enzyme loci makes it possible to detect individual strains of bacteria and provides a new and intriguing chance to study the island biogeography of man. Isolates of Escherischia coli from humans, water supplies and laboratory stocks are made up of a great number of genetically distinct and persistent clones, each of which has a unique enzyme profile12. Gene exchange among clones is a very rare event¹³ so that E. coli is in some senses a complex of a very large number of genetically well differentiated 'species' that can be identified using biochemical markers. One clone, found in 1979 in a Massachusetts infant, is identical to the laboratory strain K12, first isolated 50 years ago.

Caugant et al. 14 have studied the ecology of such clones (or species) within a Dr Bruce Levin of the University of Massachusetts. Over eleven months (which represents thousands of bacterial gener-

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ations) this human 'island' supported a fauna of 53 clones. Most were transient. and appeared on only one sampling occasion but one persisted for several months and two others occurred for most of the period of observation.

The E. coli clones therefore showed considerable 'species' turnover although the actual number of clones remained fairly constant. New clones probably arrived by immigration from the food as the nearby islands in the Levin Archipelago (the two Doctors Levin, their children and their cat) shared a similar array of clones. Clones were lost by random extinction. These observations further support the view that the species composition of islands involves considerable flux.

Biogeography and disease

Other useful analogies may be drawn between the biology of island birds and that of human pathogens. For example, islands such as the Galapagos - are uniquely favourable in promoting the origin of new bird species. It is possible that the evolution of new pathogens takes place primarily in highly sub-divided human populations and that the recent expansion of mankind to form a single large continental mass with

free interchange among its parts has greatly retarded the evolution of new disease organisms.

Birds on isolated islands with few competing species and slow species turnover have habitat preferences quite different from mainland populations². The ecology of pathogens in isolated human populations might also differ from that in modern cities.

After defaunation by volcanic explosions or poison gas, islands show changes in the diversity, turnover and ecology of their inhabitants^{17,18}; the return to equilibrium may be long delayed. Recolonization of a human host after antibiotic treatment could lead to similar and unexpected changes in the ecology of the pathogens which remain.

The most recent models of island biogeography use differences in the efficiency of migration and resource utilization of individual species to predict the species composition of birds on islands¹⁹. Clones of E. coli also differ greatly from each other in their ability to utilize resources²⁰. Developments in theories of island biogeography may therefore help to explain why some clones

are resident and some transient in the intestine¹⁴, and why certain pathogens (such as hepatitis B or herpes virus) are present in all human populations including those on islands — while others occur only sporadically8. It is even possible that such developments may help to eliminate pathogens by means of the secular miracles of medicine rather than the direct intervention of a saint.

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100 YEARS AGO

ON THE CONSERVATION OF SOLAR ENERGY¹

The question of the maintenance of Solar Energy is one that has been looked upon with deep interest by astronomers and physicists from the time of La Place downwards

The amount of heat radiated from the sun has been approximately computed by the aid of the pyrheliometer of Pouillet and by the actinometers of Herschel and others at 18,000,000 of heat units from every square foot of its surface per hour, or, put popularly, as equal to the heat that would be produced by the perfect combustion every thirty-six hours of a mass of coal of specific gravity = 1.5 as great as that of our earth.

If the sun were surrounded by a solid sphere of a radius equal to the mean distance of the sun from the earth (95,000,000 of miles), the whole of this prodigious amount of heat would be intercepted: but considering that the earth's apparent diameter as seen from the sun is only seventeen seconds, the earth can intercept only the 2,250-millionth part. Assuming that the other planetary bodies swell the amount of intercepted heat by ten times this amount, there remains the important fact that $\frac{24999999}{225000000}$ of the solar energy is radiated into

¹ Paper read at the Royal Society, March 2, by C. William Siemens, D.C.L., L.L.D., F.R.S., Mem. Inst. C.E.

space, and apparently lost to the solar system, and only $\frac{1}{2.250000000}$ utilised.

Notwithstanding this enormous loss of heat, solar temperature has not diminished sensibly for centuries, if we neglect the periodic changes, apparently connected with the appearance of sun-spots that have been observed by Lockyer and others, and the question forces itself upon us how this great loss can be sustained without producing an observable diminution of solar temperature even within a human lifetime.

Amongst the ingenious hypotheses intended to account for a continuance of solar heat is that of shrinkage, or gradual reduction of the sun's volume suggested by Helmholtz. It may, however, be urged against this theory that the heat so produced would be liberated throughout its mass; and would have to be brought to the surface by conduction, aided perhaps by convection; but we know of no material of sufficient conductivity to transmit anything approaching the amount of heat lost by radiation.

Chemical action between the constituent parts of the sun has also been suggested; but here again we are met by the difficulty that the products of such combination would ere this have accumulated on the surface, and would have formed a barrier against further action.

These difficulties have led Sir Wm. Thomson, following up Mayer's speculation, to the suggestion that the cause of the maintenance of solar temperature might be found in the circumstance of meteorolites falling upon the sun from great distances in space, or with an acquired velocity due to such fall, and he shows that each pound of matter so imported would represent a large number of heat units depending upon the original distance. Yet the aggregate of material that would thus have to be incorporated with the

sun would tend to disturb the planetary equilibrium, and must ere this have shortened our year to an extent exceeding that resulting from astronomical records and observation. In fact, Sir William Thomson soon abandoned the meteoric hypothesis for that of simple transfer of heat from the interior of a liquid sun to the surface by means of convection currents, which latter hypothesis appears at the present time to be supported by Prof. Stokes and other leading physicists of the day.

But if either of these hypotheses could be proved we should only have the satisfaction of knowing that the solar waste of energy by dissipation into space was not dependent entirely upon loss of its sensible heat, but that its existence as a luminary would be prolonged by calling into requisition a limited, though may be large, store of energy in the form of separated matter. The true solution of the problem will be furnished by a theory, according to which the radiant energy which is now supposed to be dissipated into space and irrecoverably lost to our solar system, could be arrested and brought back in another form to the sun itself, there to continue the work of solar radiation.

CAN Mr Wallace throw any light on Mr Allen's somewhat extraordinary sentence: "I feel a genuine respect for every donkey I meet. when I remember that it was the mere accidental possession of an opposable thumb that gave my ancestors a start over his in the race for the inheritance of the earth towards the very close of the tertiary period." I take Mr Allen to be an evolutionist, but there is no place for accident in evolution, or in any other scientific theory. The "opposable thumb" must be the result of some conditioning factor, and this being so the word accident is quite out

From Nature 25, 440; March 9, 1882.

REVIEW ARTICLE

Molecular mechanisms of variation in influenza viruses

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Influenza is unique among the viruses in its capacity to vary. Because of antigenic variation, it has proved impossible to control influenza by vaccination, and variation in virulence, host range and transmissibility influence the spread and severity of influenza epidemics. This variation is caused by sequence changes in the genes of the virus and this review summarizes recent information on the structure of the genes and their products, the way in which these vary and the effects of the changes on the biological activities of the virus.

OVER the past decade influenza epidemics have been relatively mild, causing only modest increases in morbidity and mortality. However, the potential of the influenza virus as a major and unpredictable threat to public health is highlighted by historic events. During the weeks from 19 October 1918 to the end of February 1919, an influenza A virus which, from circumstantial evidence, was almost certainly antigenically closely related to 'swine' influenza A virus, killed at least 20 million persons and affected perhaps one hundred times that number. It was without doubt one of the most devastating plagues ever documented. Such an event could happen again.

In 1979-80, ~20% of the harbour seal (*Phoca vitilina*) population of the north-east coast of the United States died of a severe respiratory infection with consolidation of the lungs, typical of primary viral pneumonia¹. Influenza virus particles were found in high concentrations in the lungs and brains of the dead seals. Antigenic analysis showed that this virus was closely related to fowl plague virus (A/FVP/Dutch/27 (H7N7)), a highly lethal influenza virus of chickens not previously found in mammals². What would happen if such an event occurred in man instead of seals? Would the resulting pandemic be similar to that of 1918-19?

Properties of influenza virus

Influenza viruses are classified into three types-A, B and C—on the basis of their type-specific nucleoprotein and matrix protein antigens. Type A influenza viruses are further classified into subtypes based on the antigenic characteristics of their surface antigens, haemagglutinin (HA) and neuraminidase (NA). Twelve distinct HA subtypes and nine NA subtypes are now recognized in the nomenclature system for influenza A viruses recommended by the World Health Organization³, and a thirteenth HA subtype has recently been identified⁴. Examples of the use of this nomenclature system are as follows: A/Hong Kong/68 (H3N2) is an influenza A virus isolated from man in 1968 with HA of subtype H3 and NA of subtype N2, while A/equine/Miami/63 (H3N8) is a virus isolated from horses, with HA of the same subtype as that of the human virus but with a distinct NA antigen. It is implicit in this system that all viruses with common H or N subtype have HA or NA antigens shown to be related by conventional laboratory tests (such as double immunodiffusion) while such relationships do not occur between subtypes. However, included among viruses of a common subtype designation will be strains showing considerable degrees of variation. Thus, the human virus A/Bangkok/1/79 (H3N2) contains HA and NA antigens demonstrably related to those of A/Hong Kong/68 virus but showing considerable degrees of difference from them. During

the present century three distinct subtypes of influenza A virus have caused pandemics in man—the H1N1 subtype which probably appeared in 1918, the H2N2 ('Asian') subtype which appeared in 1957 and the H3N2 ('Hong Kong') subtype which appeared in 1968.

This review will be concerned with type A influenza, which has been responsible for the major pandemics and has been investigated more extensively than types B and C.

The highly pleomorphic particles of influenza virus are enclosed by a lipid envelope, derived from the plasma membrane of the host cell, to which the HA and NA antigens are attached by short sequences of hydrophobic amino acids at one end of the molecules⁵⁻⁷. Both surface antigens are glycosylated and some of the carbohydrate side chains possess antigenic activity characteristic of the host cell in which the virus grew.

Within the lipid envelope lies the matrix protein, which is believed to be structural in function. Within the matrix shell are eight single-stranded RNA molecules of negative sense (that is, the virion RNA is complementary to the messenger RNA) associated with a nucleocapsid protein and three large proteins, P1, P2 and P3, responsible for RNA replication and transcription. At least three virus-encoded non-structural proteins are found in infected cells, but their functions are unknown⁸⁻¹⁰. Table 1 summarizes current information on the eight genes of the influenza virus and the molecular weights (M_r) of the proteins encoded by them.

Variation in haemagglutinin

The HA accounts for about 25% of the virion protein. It is responsible for attachment of the virus to cells and for the stimulation of antibodies that neutralize the virus. The HA monomer is synthesized as a single polypeptide chain which undergoes post-translational cleavage at three places. An N-terminal signal sequence is removed and, depending on the host cell and virus strain, the molecule is cleaved, with the removal of one or more intervening residues, to give two polypeptide chains called HA1 and HA2, with molecular weights of 36,000 and 27,000, respectively (Table 1). A sequence of hydrophobic amino acids near the C-terminus of HA2 serves to anchor the HA in the virus membrane.

The three-dimensional structure of Hong Kong (H3N2) HA has recently been determined¹¹ and it seems likely that HA molecules from other subtypes will have similar structures. The HA glycoprotein of influenza virus is a trimer built of two structurally distinct regions: a triple-stranded coiled-coil of α -helices extend 76 Å from the membrane and a globular region of antiparallel β -sheet that contains the receptor binding site; the variable antigenic determinants are located on top of this

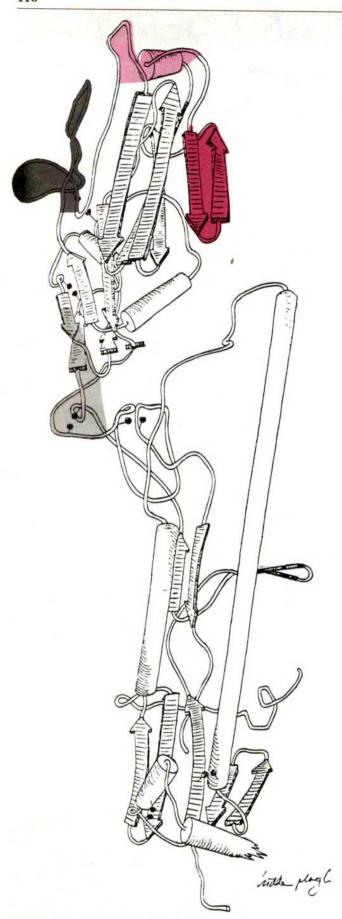


Fig. 1 Drawing (by Hidde Pleogh) of the Hong Kong (H3) HA monomer showing folding of the HA1 and HA2 polypeptides^{11,12}. The four coloured areas show where four independent antigenic areas may be located. Note that in the HA spike, which is composed of three of the monomers, the dark red site is buried and may not be involved in antibody binding.

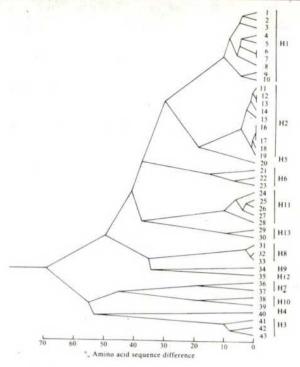


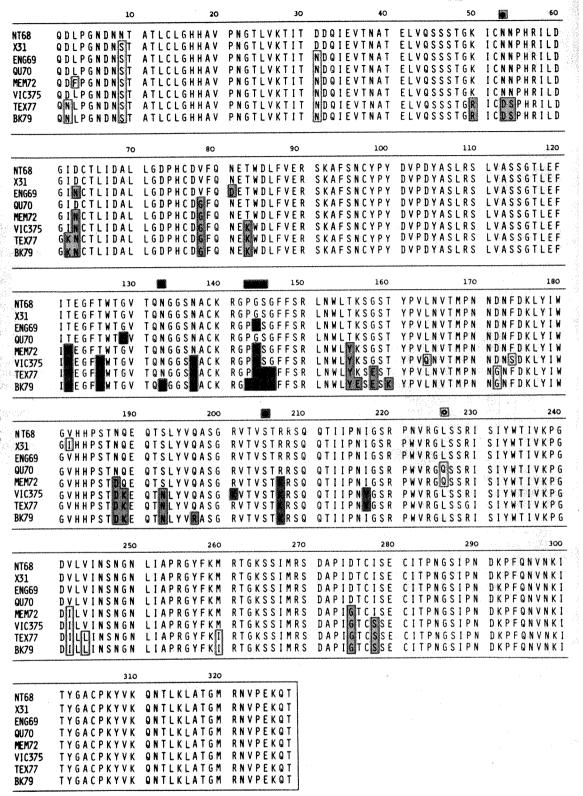
Fig. 2 Dendrogram showing the relationships between the HA1 Nterminal amino acid sequences deduced from genomic sequences of viruses representing the 13 known subtypes. The sequences were aligned using amino acids that are invariable in certain positions of all sequences (amino acid position from N terminus of most subtypes: Cys 4, Gly 6, Thr 18, Val 26, Cys 42, Cys 55, Gly 63, Pro 65, Cys 67, Glu 81). The percentage sequence differences of all pairwise comparisons of the aligned sequences starting from the N-terminal Asp or corresponding amino acid were used to calculate the dendrogram. Thus, the positions of each bifurcation in the dendrogram indicate the mean sequence difference of the sequences connected through that point⁴. The sequences used in the analysis are: 1, A/NWS/33 (H1N1); 2, A/PR/8/34 (H1N1); 3, A/BH/35 (H1N1); 4, A/Bel/42 (H1N1); 5, A/Loyang/4/57 (H1N1); 6, A/USSR/90/77 (H1N1); 7, A/Fort Warren/1/50 (H1N1); 8, A/duck/Alberta/35/76 (H1N1); 9, A/swine/Wisconsin/15/30 (H1N1); 10, A/New Jersey/11/76 11, A/RI-/57 (H2N2); 12, A/Tokyo/3/67 (H2N2); 13, eley/68 (H2N2); 14, A/Netherlands/68 (H2N2); 15, A/duck/GDR/72 (H2N9); 16, A/duck/New York/6750/78 (H2N2); 17, A/duck/Ontario/77 (H2N1); 18, A/duck/Alberta/77/77 (H2N3); 19, A/duck/Ollaino/// (H2N1); 16, A/duck/Alberta///// (H2N3); 17, A/pintail/Alberta/293/77 (H2N9); 20, A/shearwater/Australia/75 (H5N3); 21, A/shearwater/Australia/72 (H6N5); 22, A/duck/Alberta/1298/79 (H6N5); 23, A/duck/Alberta/1297/79 (H6N9); 24, A/tern/Australia/75 (H11N9); 25, A/duck/Ukraine/1/60 (H11N9); 26, A/duck/England/56 (H11N6); 27, A/duck/New (H1N9); 24, A/tern/Australta/75 (H1N9); 25, A/duck/Ukraine/1/60 (H1N9); 26, A/duck/England/56 (H1N6); 27, A/duck/New York/12/78 (H1N6); 28, A/duck/Memphis/576/76 (H1N9); 29, A/gull/Mass/26/80 (H13N6); 30, A/gull/Md/704/77 (H13N6); 31, A/turkey/Ontario/6118/68 (H8N4); 32, A/duck/Alberta/827/78 (H8N4); 33, A/duck/Alberta/283/77 (H8N4); 34, A/turkey/Wisconsin/1/66 (H9N2); 35, A/duck/Alberta/60/76 (H12N5); 36, A/duck/Olara (Table 1) (H12N5); 36, A/duck/Olara (sin/1/66 (H9N2); 35, A/duck/Alberta/60/76 (H12N5); 36, A/turkey/Oregan/71 (H7N3); 37, A/equine/Prague/1/56 (H7N7); 38, A/duck/Manitoba/53 (H10N7); 39, A/quail/Italy/1117/65 (H10N8); 40, A/duck/Alberta/28/76 (H4N6); 41, A/black duck/Australia/702/78 (H3N8); 42, A/Memphis/1/71 (H3N2); 43, A/duck/Ukraine/1/63

stem. Each subunit has an unusual loop-like topology: it begins at the membrane, extends 135 Å distally and folds back to enter the membrane.

Four antigenic sites on the three-dimensional structure of the HA molecule of Hong Kong influenza viruses have been proposed ¹²⁻¹⁴. The suggested locations (Fig. 1) were derived from amino acid sequence changes in antigenic variants of Hong Kong virus selected using monoclonal antibodies ¹⁵ and in natural variants of this virus ^{11,16,17}.

Variation in the HA molecule of influenza viruses occurs in two ways. It is now known that small changes in antigenicity (antigenic drift) are the result of a gradual accumulation of point mutations, while the complete change in antigenic properties (antigenic shift) involves replacement of the gene coding for one HA with that for another; this may or may not be accompanied by replacement of the NA gene.

Fig. 3 Amino acid sequences using the single letter amino acid code of the HA1 polypeptide from eight variants of Hong Kong (H3N2) influenza virus isolated between 1968 and 1979. Data are from refs 17, 21, 25, and B. A. Moss and G Brownlee (unpublished results). Residues which have changed from NT68 are coloured or boxed. The location of most of these changes on the threedimensional structure of the HA are shown in Fig. 1. (Boxed changes were scatthe threetered over dimensional structure and may not affect the antigenic properties of the HA. They have not been shown in Fig. 1.) •, The sequence changes found in selected with variants monoclonal antibodies¹⁵. These are (53) Asn→Lys, Asn → Lys, (143) (133)Pro → Ser, Thr, Leu, His, (144) Gly → Asp, Ser → Lys, (205) Ser → Tyr. The change (226) Leu→ Gln (O) appears to arise with remarkable ease. This change has been found in different clones of the same field strain and in monovariants clonal which already show another may change and he unrelated to changes antigenicity.



Antigenic shift

Since the first human influenza virus was isolated in 1933, antigenic shifts have occurred in 1957 when the H2N2 subtype (Asian influenza') replaced the H1N1 subtype, in 1968 when the Hong Kong (H3N2) virus appeared and in 1977 when the H1N1 virus reappeared. All these major antigenic shifts in the virus occurred in China and anecdotal records suggest that previous epidemics (probably caused by the sudden emergence of 'new' viruses) also had their origin in China. Although we are still uncertain how these new subtypes appear or reappear in the human population, some clues are emerging from the plethora of recent sequence data.

The complete amino acid sequences of HA1 and HA2 have been deduced from the sequences of cloned DNA copies of the genes for strains representative of four different HA subtypes of type A influenza—fowl plague (H7N7) virus¹⁸, Asian (H2N2) influenza virus⁶, PR8 and WSN (H1N1) viruses^{19,20} and a number of strains within the human Hong Kong (H3N2) subtype^{17,21}. A complete protein sequence analysis is available only for the H3 strain^{22,23}, although sufficient protein data are available for the H2 strain to indicate where the signal peptide is removed and where the molecule is cleaved into HA1 and HA2. The data also show the location of the single disulphide bond connecting HA1 and HA2 and that most of the potential

glycosylation sites in the HA do have carbohydrate attached. The complete sequences of two strains of A/Duck/Ukraine/63 (H3N8) haemagglutinin have also been determined, one from gene sequence data²⁴, the other from direct protein sequencing²⁵.

Sequences up to 350 nucleotides from the 3' end of the HA gene and the predicted amino acid sequences from 32 type A influenza viruses, including representatives of each of the 12 known HA subtypes, have been determined²⁶. Cysteine residues and certain other amino acids are conserved in all sequences, indicating that the 12 HA subtypes evolved from a common ancestor and share a common basic structure. When the partial amino acid sequences are compared pairwise, the most distant subtypes are H1 and H3 (25% homology). Other subtypes are more similar to each other, the highest homology (80%) being between H2 and H5. Relationships between the partial sequences can be illustrated as a dendrogram (Fig. 2).

Within strains of a single subtype, the differences in deduced amino acid sequence are generally less than 10% (ref. 26), but recent extensions of the data have revealed differences in the N-terminal region of HA1 from two strains each of H7 and H10 viruses close to 20%, almost equal to the difference between the amino acid sequences of the two closest subtypes (H2 and H5).

Whereas it is possible to imagine that the H2 viruses could eventually mutate into H5, the change to H3 must involve a more drastic mechanism. How, then, could the H3 haemagglutinin have replaced the H2 strain in the human population in 1968? First, the 'new' virus may have caused an epidemic in man many years previously and have remained hidden and unchanged in some unknown place ever since. Evidence for this kind of event has been obtained. The strain of 'Russian 'flu' (H1N1) which reappeared in Anshan in northern China on 4 May 1977 and subsequently spread to the rest of the world, seems to be identical, in all genes, to the virus which caused an influenza epidemic in 1950^{27,28}. Where was this virus for 27 years? As yet we have no answer.

Second, some of the 'new' viruses may be derived from animal or avian viruses. One strain of human influenza has been shown to be such a recombinant (reassortant)²⁹⁻³¹. The Hong Kong (H3N2) virus contains the NA (and other) genes from an Asian (H2N2) strain of human influenza and a HA which is antigenically related to that of A/Duck/Ukraine/63 (H3N8) and A/equine/2/Miami/63 (H3N8) viruses^{24,25}. The amino acid sequence homology between the HAs of A/Duck/Ukraine/63 and A/Aichi/2/68 viruses (both of subtype H3) is 96%. We do not know if it was an animal or bird virus which donated the HA gene during the recombination event that led to the formation of the Hong Kong strain. The donating virus could have been one which contained Hong Kong HA that had persisted from a much earlier human influenza epidemic maintained unchanged in the same way as the Russian 'flu. Antibodies in the sera of people who were born around 1900 suggest that a virus with a HA similar to that of the Hong Kong virus caused influenza at that time³², but the NA of this virus was N8, not N2. It is possible that viruses such as equine (H3N8) and duck/Ukraine have evolved from the 1900 epidemic strain.

The third way in which new viruses could appear in the human population is if an animal or bird virus became infectious for man. This could be brought about by mutation, but the number of mutations required and the genes in which these would need to occur are unknown.

Perhaps each of these mechanisms has operated at one time or another to produce a 'major shift' in influenza viruses infecting man. We do not know how the different subtypes of type A influenza arose, but it is reasonable to suppose that 'shuffling' of genes between influenza A strains is occurring all the time, most frequently in birds³³, but also in mammals, including man^{34,35}.

Antigenic drift

Antigenic drift in the HA occurs by mutations in the gene, leading to an accumulation of amino acid sequence changes that alter the antigenic sites in such a way that they are no longer

recognized by the host's immune system. The evidence for this mechanism comes from sequence analysis of naturally occurring 'drifted' viruses (mainly those of the Hong Kong (H3N2) virus) and of variants selected in the laboratory in the presence of antibodies. Most of the sequence changes occurring in Hong Kong HA between 1968 and 1979 were in the HA1 portion of the HA molecule, only three changes being found in HA2¹⁷. The sequence changes in HA1 during this period are shown in chronological order of isolation of the strains in Fig. 3. Although these changes are distributed over HA1, on the three-dimensional structure of the HA many are clustered into four regions (Fig. 1) which are assumed to be the antigenic sites on the HA. Sequential changes at a particular position in HA1 have not occurred. A few apparent reversions were seen, but as there is no reason to believe that the viruses examined represent a direct genealogical lineage, it is likely that in those cases in Fig. 3 where there are apparent reversions of an amino acid, the real explanation is that the residue never changed in the first place. Similarly, in the one case where there is an apparent sequential change in an amino acid (the asparagine residue at position 137 changing to serine in Vic/75 and then to tyrosine in Bangkok/1/79), the nucleotide changes involved indicate that the Vic/75 strain was not the progenitor of the Bangkok/1/79 strain.

Some regions of HA1 have been totally conserved during the period 1968–79. The largest of these conserved regions occurs between residues 84–121 and 279–328. The latter block is also conserved in the two avian H3 strains so far sequenced^{24,25}, except for a single change of valine to isoleucine at position 309. Residues 279–328 form part of the 'stalk' of the HA where structural constraints may necessitate that they are highly conserved.

Variants selected with monoclonal antibodies

Antigenic variants of the HA have been selected by growing virus in the presence of monoclonal antibody to the HA. The variants occurred at a frequency of $\sim 10^{-5}$ in virus stock^{36,37} and did not bind at all to the antibody used for their selection. This dramatic change in antigenicity was found to be associated with single changes in the amino acid sequence of HA1^{15,37} (see Fig. 3). Most antigenic variants of Mem/1/71 (H3N2) selected with monoclonal antibodies could not be distinguished from parental virus with antisera from ferrets infected with the parent strain, but two could be distinguished¹³. Some antigenic variants of B/Hong Kong/1/73 could also be discriminated³⁸. Presumably, their amino acid substitutions (Fig. 3) cause more profound changes in the antigenicity of the molecule than others. It seems likely that only variants which have such profound changes have sufficient selective survival advantages in an immune population to produce an epidemiological impact.

Variation in neuraminidase

Neuraminidase is an integral membrane protein in influenza virus and can be solubilized with detergents or pronase³⁹. The detergent-soluble NA has a molecular weight^{40,41} of ~240,000 and consists of four identical polypeptide chains arranged in such a way that the molecule possesses a hydrophilic 'head' attached to the hydrophobic membrane-insertion sequence by a thin stalk. The active centre of the enzyme and the antigenic determinants are distinguishable from each other⁴². Pronase digestion of the virus releases the soluble NA head of M_r ~200,000 which retains the antigenic and enzymatic activity of the intact molecule. The NA heads of some strains of influenza virus can be crystallized⁴³ and determination of the three-dimensional structure of A/Tok/3/67 (H2N2) NA is in progress⁴⁴. The Fab fragment from a monoclonal antibody to A/Tok/3/67 (H2N2) NA has also recently been crystallized and the complementary surfaces of this antigen-antibody complex can now be mapped⁴⁵.

The complete sequence of the NA gene of A/PR/8/34 has been determined⁴¹ and from the predicted amino acid sequence and analysis of intact NA and NA heads of N2, N5 and N8

Table 1 Influenza virus-coded proteins

	Approximate no. of molecules		Molecular w	eight estimated by			
Designation	per virus particle	Gel electrophoresis	Gene sequence*	Gene + protein sequence	Ref.	Remarks	
P1 polymerase 1 P2 polymerase 2 P3 polymerase 3 HA haemagglutinin	} 30–60	96,000 87,000 85,000			91 91 91	Internal proteins associated with RNA transcriptase activity.	
HA1				36,074+11,500 (Mem/102/72)†	22,84	Surface glycoprotein responsible for attach- ment of virus to cells.	
H A 2				27,368+1,400 (Mem/102/72)†	23,84	Trimer composed of two polypeptides HA1 and HA2 formed by post-translational cleavage of the primary translation product. Three-dimensional structure known.	
NP nucleoprotein	1,000	50-60,000	56,106		55	Internal protein associated with RNA and polymerase proteins, helical arrangement.	
NA neuramınıdaşe	100	48–63,000	50,087		41	Surface glycoprotein, with enzyme activity. Tetragonal molecule with a 200,000-M _r , head—crystallized and structure in progress.	
M1 matrix	3,000			27,861	58, 59, 62	Major virion component surrounding the core, involved in assembly and budding.	
M2 matrix		15,000	11,000		10	Coded from the same gene segment as M1 in a second reading frame, a non-structural protein, function unknown	
NS1 non-structural protein		25,000	26,815		64	Non-structural protein, function unknown.	
NS2 non-structural protein	ı	12,000	14,216		64	Non-structural protein coded for in a second reading frame from same gene segment as NS1, function unknown, synthesized late in infection	

^{*} These values are from the predicted amino acid sequence and do not take into account any processing the final product may have undergone

subtypes^{7,46} it has been found that, unlike the HA, the NA is oriented with its N terminus buried in the viral membrane and does not have a signal peptide which is cleaved.

Antigenic shift and antigenic drift occur in the NA; among the human influenza viruses antigenic shift occurred in 1957, with the emergence of the H2N2 subtype and the replacement of the N1 by N2. Antigenic drift in the NA of influenza viruses⁴⁷⁻⁴⁹ has been correlated with differences in amino acid sequences^{43,50}.

Partial sequences up to 340 nucleotides from the 3' end of the NA gene have been obtained for representative viruses from eight of the nine NA subtypes⁵¹. These sequences will correspond to the region encoding the N-terminal part of the NA since analysis of tryptic peptides shows that translation begins from the first AUG codon and that no signal peptide is cleaved from the N terminus. The first six amino acids are conserved in all viruses of all subtypes examined and the next six are conserved in most subtypes. Following these, the nucleotide and predicted amino acid sequences of the eight subtypes of NA differ dramatically in the hydrophobic membrane insertion sequence and the extended structure of the stalk. Deletions and/or insertions of short blocks of nucleotides in the NA gene are seen within subtypes⁵².

Antigenic variants of the NA have been selected using monoclonal antibodies at a frequency of ~ 1 in 10^5 (ref. 53), similar to the frequency of variants in the HA molecule³⁶. Analysis of antigenic variants with a panel of N2 monoclonal antibodies provided evidence for at least three and possibly four non-overlapping antigenic areas on the NA molecule of A/Tokyo/3/67⁵³. Studies on natural antigenic drift indicate that it occurs by the same mechanisms as in the HA, with a limited number of base changes in the NA gene within subtypes⁵⁴.

Variation in the nucleoprotein

The nucleoprotein (NP) is one of the group-specific antigens of influenza viruses that distinguishes between the influenza A, B and C viruses. It probably constitutes the backbone of the helical internal complex that is associated with the RNA segments and the three different polymerases.

The NP gene of A/PR/8/34 virus is 1,565 nucleotides long and is capable of encoding a protein of 498 amino acid residues

(M_r56,106) rich in arginine⁵⁵. Double immunodiffusion tests showed antigenic differences between the NPs of H1N1 and the H3N2 strains⁵⁶ and recent studies with monoclonal antibodies to the NP of A/WSN/33 (H1N1) viruses have shown that antigenic variation occurs in this molecule⁵⁷. The NP molecule possesses at least three non-overlapping antigenic areas, one area being the same on all strains tested. Monoclonal antibodies to this conserved domain inhibited *in vitro* transcription of viral RNA, suggesting that this region of the NP is involved in RNA transcription⁵⁷.

The matrix protein

The complete sequence of RNA segment 7 (M) of two strains of A/PR8/34 (H1N1)^{58,59} and of A/Udorn/72 (H3N2)⁶⁰ has been reported as well as partial sequences of a number of strains⁶¹. Following the first AUG codon in the positive strand, a 252-residue protein, rather hydrophobic and rich in arginine, is encoded. Compositions of tryptic peptides from purified matrix protein of PR8⁶¹ correspond well with those expected from the amino acid sequence predicted from the gene sequence.

Comparison of the sequences of RNA segment 7 of the H3N2 (Udorn) and H1N1 (PR8) strains shows that the sequences coding for the matrix (M) of these viruses isolated 38 years apart are highly conserved⁶⁰, in keeping with antigenic studies⁶². Comparison of 230 nucleotides of RNA segment 7 from five human H1N1, H2N2 and H3N2 strains isolated over a 43-yr period suggests that the same segment 7 was retained throughout the antigenic shifts of HA and NA type (H1N1 to H2N2 to N3N2)⁶¹. In addition, the complete sequences contain a second open reading frame which overlaps the M protein sequence by 68 nucleotides.

Three mRNAs transcribed from RNA segment 7 have been isolated. One (M1 mRNA) consists of an uninterrupted, nearly full-length, copy of RNA segment 7 and is responsible for the production of the M protein. An M2 protein is generated from a spliced product of the M1 mRNA, such that after the nucleotides encoding the N-terminal nine amino acids, nearly 600 nucleotides are spliced out and the reading frame is changed; a protein product corresponding to this has been identified in infected cells¹⁰. In addition, a third mRNA has been found, which would code only for a 8-residue peptide identical to the C terminus of M1⁶³. Such a product has not yet been isolated.

[†] The second value is the carbohydrate contribution.

The non-structural proteins

Recent studies have shown that RNA segment 8 codes for at least two non-structural polypeptides, NS1 and NS2, which are translated from separate mRNAs⁶⁴⁻⁶⁶. Mapping and sequence studies have shown that NS1 and NS2 overlap by 70 amino acids that are translated from different reading frames. Polypeptides NS1 and NS2 share 9 amino acids at their N termini, but after this sequence the mRNA for NS2 has a deletion of 423 nucleotides, then rejoins the rest of the mRNA in the +1 reading frame. The function of NS1 or NS2 has not been established. NS1 is made in large amounts and accumulates in the nucleus⁶⁷; NS2 is made late in infection and is found predominantly in the cytoplasm^{64,68}.

Because NS1 and NS2 are internal proteins of infected cells and hence less available to antibodies, they would be expected to show less sequence variation than the surface glycoproteins (HA and NA). Accordingly, comparison of the sequences of the NS genes of fowl plague⁶⁵ and the two human influenza strains A/Udorn/72 (H3N2) and A/PR/8/34 shows only 8-11% differences^{64,69,70}. An open reading frame potentially coding for a polypeptide has been noted in the noncoding, virion RNA of the NS genes of A/PR/8/34, Udorn/72 and FPV, but is not present in the NS gene of duck/Alberta/60/76⁷¹. No protein corresponding to this extra 'gene' has yet been identified.

Polymerase proteins

The three largest proteins of the virion (P1, P2, P3) with M,s of 96,000, 87,000 and 85,000, respectively, are found in association with the nucleoprotein and virion RNA and carry the polymerase activity⁷² which transcribes the invading viral RNA⁷³. Proteins P1 and P3 are probably required for complementary RNA synthesis and P2 and NP for virion RNA synthesis⁷⁴.

Transcription of the viral RNA segments soon after infection terminates ~40 nucleotides before the 5' end to give messenger RNAs⁷⁵; these mRNAs have a cap structure and a heterogeneous sequence of 10–13 nucleotides derived from cellular mRNAs at their 5' end⁷⁶. Later transcripts are full length and act as templates for synthesis of the genes of the progeny virus. The complete nucleotide sequences of two of the three polymerase genes of the A/PR/8/34 strain have been determined⁷⁷, but the extent of variation in the polymerase genes is unknown, although these may play an important part in host range and virulence.

Defective interfering (DI) influenza virus particles are generated by high multiplicity passage in permissive cells. These particles are of interest because they facilitate the establishment of persistent infection in cell cultures and could therefore be concerned in latency. They contain new small RNA molecules which are absent from infectious virus and which are generated predominantly by massive internal deletion from the three P genes^{78,79}, although the sequences of one small RNA is a mosaic of several segments from at least two of the polymerase genes^{77,80}. This is the first time intragenic recombination has been shown in influenza virus.

Variation in pathogenicity

Highly pathogenic avian influenza viruses that cause generalized infections and death in avian species exist among viruses of the H7 (formerly Havl) and H5 (Hav5) HA subtypes, while other viruses of these subtypes are relatively non-pathogenic although fully infectious. Efficient cleavage of the precursor HA polypeptide into HA1 and HA2 in a wide range of cells from different tissues appears to be essential for the pathogenicity of these viruses⁸¹⁻⁸³. The amino acid sequence of the connecting peptide between HA1 and HA2 may determine the range of cells in which cleavage can occur. Fowl plague virus, which produces almost invariably lethal infections in chickens, has the sequence Lys-Lys-Arg-Gln-Lys-Arg between the C terminus of HA1 and the N terminus of HA2¹⁸, while the human viruses, which are avirulent in chickens, and non-pathogenic avian viruses of the same subtype as fowl plague (H7), contain only a

single connecting arginine residue ^{6.17,22,23,84}. Investigation of groups of pathogenic and non-pathogenic avian influenza viruses of the H7 subtype suggested that the HA molecules from the non-pathogenic viruses had fewer basic residues in their connecting HA peptides than the pathogenic strains⁸⁵.

Examination of the amino acids of the amino-terminal sequence of HA2 of several influenza A viruses of human and avian origin has revealed a highly conserved region of 10 residues ^{86,87}. This sequence is homologous ⁸⁸ to the amino-terminus region of the fusion glycoprotein F, of Sendai virus (a paramyxovirus), which mediates fusion of the Sendai virus envelope with the plasma membrane of host cells, a function which is considered essential for virus infectivity. It has been postulated, therefore, that the infectivity of influenza virus is dependent on a fusion function of HA analogous to that of the Sendai fusion protein. Although the three-dimensional structure of the influenza HA shows that this peptide is buried in the molecule, it has recently been shown that after incubation in conditions required for membrane fusion, that is, at pH 5.0, a conformational change occurs in the influenza HA glycoprotein and the molecule acquires the ability to bind to lipid vesicles ⁸⁹.

However, the structure of the HA is almost certainly not the only factor determining pathogenicity. While reassortment studies between highly virulent and avirulent viruses pointed to the prime importance of the HA for pathogenicity, they also showed that the polymerase (P3) and nucleoprotein genes could be involved. Other experiments have shown that a specific gene constellation, rather than any single gene, is responsible for the pathogenic properties.

What of the future?

The recent explosion of structural data on the genes and gene products of influenza viruses has provided some important information about the behaviour of this virus. For example, it is clear that antigenic shift does not occur by direct mutation of one subtype into another, while antigenic drift occurs by point mutation in the genes. However, the mutation frequency of the surface proteins is not significantly greater than that of the internal proteins. HA possesses at least four distinct antigenic sites which can vary independently, with pathogenicity depending in part on the sequence in the HA where cleavage occurs. Some RNA segments of the virus possess overlapping genes, using two reading frames. The sequence data support the antigenic classification of the HA and NA subtypes.

However, many mysteries remain. Shifts can occur by the emergence of viruses which previously caused epidemics and have remained unchanged, as if frozen, for many years. Where these viruses hide, and what causes them to re-emerge, is a complete mystery. The shifts may also occur by genetic reassortment of human and animal influenza viruses or by mutation of an animal or avian virus so that it becomes able to infect people. Most type A influenza virus subtypes are found in birds, but the role of these in the emergence (or re-emergence) of new subtypes in man is not clear; nor is it clear why the shifts always occur in China or why the old subtypes usually disappear from man as the pandemic of new virus starts. On the other hand, the Hong Kong subtype did not disappear when Russian 'flu started and we do not know why this was.

What is the origin of the various subtypes? How many subtypes are there in birds and animals as well as in man? Can all subtypes of HA and NA be incorporated into viruses infecting man? Why are the antigenic shifts restricted to type A influenza? What triggers the appearance of a new subtype?

It is not known precisely how the amino acid sequence changes which occur in the HA and NA during drift alter the antigenic properties. Does drift follow a set path and can drift be predicted? Why do few, if any, sequential changes at particular positions in the HA molecule occur?

Are there particular areas on the HA and NA which induce neutralizing antibody while others do not? Does drift involve changes in the immunogenicity of different antigenic determinants? The location on the HA molecule of the sequence changes which occur during antigenic variation in the H3 subtype is known, but whether the amino acids in these areas make contact with the combining site on antibody molecules is not.

Does drift continue indefinitely? Can it go backwards? It is not known if the same antigenic change occurs simultaneously in different areas of the world or whether a single mutant arises somewhere and all spread derives from a single focus. Why does antigenic drift occur in influenza, but not, for example, in paramyxoviruses such as measles virus, where antigenic variants can be selected *in vitro* with monoclonal antibodies at about the same frequency as influenza^{92,93}?

Why does one sometimes observe 'original antigenic sin', in which a virus may preferentially elicit antibodies against a previous strain which has since disappeared? In addition to antigenic variation, variation in host range, virulence and transmissibility greatly influence the severity of the disease and the spread of epidemics. Is it possible that a virus could appear which would cause the same degree of mortality as the 1918 'swine' 'flu epidemic? What influences the spread of influenza virus, and why do epidemics normally appear in the winter, thus oscillating between the Northern and Southern Hemispheres? It is not known why epidemics are self-limiting, even when susceptible hosts remain in abundance.

There are now, however, better prospects for the control of influenza. Effective and safe live vaccines may be made in the future using modern genetic engineering techniques. Synthetic vaccines may be possible where the antigens are pure enough for the vaccines to be given in large enough doses to be effective and engineered such that they do not induce original antigenic sin. A universal vaccine, which protects against all members of a

subtype or even across subtypes might be feasible. It might also be more rewarding to look for ways of increasing the cellular immune response as well as the humoral response.

With the three-dimensional structure of at least one HA

With the three-dimensional structure of at least one HA molecule known and candidates for the antigen and receptor binding sites tentatively identified, the way is open for the synthesis of vaccines containing key segments of HA molecules. HA antigenic determinants can be detected in *Escherichia coli* transformed with a plasmid carrying a cloned HA gene^{94–96}, and copious amounts of HA appear on the surfaces of eukaryotic cells infected with a simian virus 40 (SV40) vector containing the HA gene^{97,98}. These experiments open the way to the use of site-directed mutagenesis to tailor the HA into a successful synthetic and possibly universal vaccine.

What of the immediate future? We do not know if another antigenic shift is imminent. If a new virus does suddenly appear, will the structural data tell us where it came from? The new virus might not have the capacity to kill people in the same way as the recent seal virus killed seals, but if it did, it is doubtful that we would be able to stop it causing world-wide devastation. If no shift occurs, what will happen to the currently circulating H3N2 and H1N1 strains? Will they continue to drift, or have they reached their limit? There is little doubt that a greater understanding of the molecular biology and genetics of the virus will make an essential contribution in piecing together the answers to the puzzle of influenza.

The authors were supported in part by grants AI08831, AI02649 and AI15343 from the National Institute of Allergy and Infectious Diseases. The writing of this joint review was greatly assisted by international telephone facilities donated by the Australian Overseas Telecommunications Commission.

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ARTICLES

Revisions of K/Ar ages for the Hadar hominid site, Ethiopia

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Conventional K/Ar age measurements are reported for two volcanic units from the hominid-bearing Hadar Formation. These ages represent revision of previously published ages for the BKT-2 tephra and the Kadada Moumou basalt. BKT-2 is revised from 2.65 to 2.9 Myr BP and the Kadada Moumou basalt is revised from 3.0 to 3.6 Myr BP.

PREVIOUS isotopic age determinations for the Hadar Formation relied on two volcanic horizons, the BKT-2 tephra in the upper Kada Hadar (KH) Member and the Kadada Moumou basalt (KMB) in the upper Sidi Hakoma (SH) Member (Fig. 1). New work has focused on additional dating of these two units as well as on many of the other tuffs in the formation that are less adequate for dating¹. This article presents revised geochronology for the BKT-2 tuff and of the Kadada Moumou basalt. The revisions are based on three components: (1) new abundance and decay constants for ⁴⁰K that are now widely accepted²; (2) new values for the ³⁸Ar internal standard used in the study; and (3) running of additional samples that have shed new light on the age interpretation. These considerations cause us to propose an increased age for these units.

New decay and abundance constants for 40K

In August 1977, the International Union of Geological Sciences (IUGS) Subcommission on Geochronology proposed a revision of the decay constants and the atomic abundance of 40 K (ref. 2). The new constants are $\lambda_{\star}=0.581\times10^{-10}~\text{yr}^{-1},~\lambda_{\beta}=4.962\times10^{-10}~\text{yr}^{-1},~\text{and}~^{40}\text{K/K}=1.167\times10^{-4}.$ Plio–Pleistocene K/Ar ages calculated with the old constants are increased by $\sim\!2.7\%$ when recalculated with the new constants³. Therefore, previously published K/Ar ages for the Pliocene Hadar Formation $^{4-6}$ must be categorically increased by 2.7%.

Revision of CWRU ³⁸Ar pipette constants

The K/Ar laboratory at Case Western Reserve University (CWRU) utilizes a bulb $^{38}{\rm Ar}$ tracer system with an all-metal, two-valve gas pipette machined in Heidelberg after the type used at the Max Planck Institute. The bulb was filled with the $^{38}{\rm Ar}$ residue remaining after a commercial preparation of $^{38}{\rm Ar}$ batch tracers. A discrepancy arose between the pipette volume calculated from the amount of $^{38}{\rm Ar}$ stipulated for the initial fill by the commercial glassblower and the pipette volume measured by the machinist who made the pipette. In our previous reports on the Hadar Formation $^{4-6}$ the 'calculated' rather than the 'measured' volume was used and we reported an average of $18.4\times10^{-10}\,{\rm mol}\,{\rm g}^{-1}$ of $^{40}{\rm Ar}^*$ (radiogenic argon) for the LP6 interlaboratory biotite standard. This value is significantly lower than the accepted LP6 $^{40}{\rm Ar}^*$ yield of $19.3\times10^{-10}\,{\rm mol}\,{\rm g}^{-1}$ (J. C. Engles, personal communication).

We have now pipetted over 1,000 aliquots from the bulb system and monitored its depletion against 35 analyses of the LP6 standard (Table 1). The depletion behaviour (Fig. 2) is only explained by the machinist's measurement of the pipette volume (method B) and not by the glassblower's calculation of the initial $^{38}\mathrm{Ar}$ content (method A). Thus we have recalibrated our $^{38}\mathrm{Ar}$ internal standard to the measured pipette volume and recalculated the initial $^{38}\mathrm{Ar}$ fill to correspond to the accepted value of 19.3×10^{-10} mol g $^{-1}$ of $^{40}\mathrm{Ar}^*$ for the LP6 biotite standard.

Table 1 shows our results for several inter-laboratory standards including LP6 biotite, MMhb hornblende (University of Minnesota), SB biotite (US Geological Survey), Bern 4M muscovite and Bern 4B biotite and Berkeley African Feldspar: (BAFIS) A, B and C (ref. 7 and personal communications from J. C. Engles, M. Lanphere, and E. Jager).

In Tables 2 and 5 all previously published K/Ar ages for the Hadar Formation have been corrected for the recalibrated ³⁸Ar pipette constant as well as the new radiometric and abundance constants.

Additional samples and new measurements

BKT-2, believed to be a primary ash-fall tuff, overlies nearly all of the fossil vertebrate fauna of the Hadar Formation⁵ (Fig. 1). The tuff commonly occurs as a doublet horizon, with the upper ash layer (BKT-2u) separated from the lower ash layer (BKT-2L) by 0.5-1.0 m of fluvial overbank silty clay⁸.

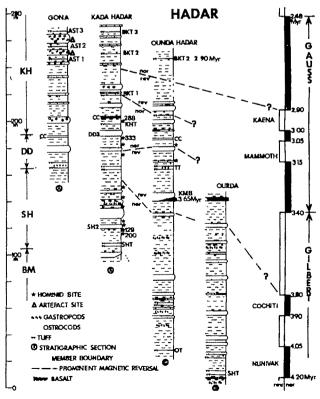


Fig. 1 Stratigraphical sections proceeding from west (left) to east (right) across the Hadar Site (taken from ref. 8) Location of sections shown in Fig 3. Prominent magnetic polarity reversals and their possible correlation to the worldwide reversal time scale (ref. 3) are indicated.

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Table 1 Interlaboratory K/Ar standards

	% K ₂ O		⁴⁰ Ar* (10 ⁻¹⁰ r	No. of	
Sample	Measured	Accepted	Measured	Accepted	measurements
LP6 biotite†			19.25 ± 0.56	(19.30)	35*
MMhb hornblende ⁷	1 89	1.874	16.31 ± 0.10	(16.24 ± 0.09)	4
SB biotite‡	9.188	9.188	22.78 ± 0.11	(22.45 ± 0.29)	3
Bern 4M muscovite§	10 58	10.48	2.716 ± 0.015	(2.82)	4
Bern 4B biotite§	9.57	9.52	2.446 ± 0.014	(2.38)	2
Berkeley African feldspar A	4.725	(¶)	0.1233 (1.81 Myr BP)	(¶)	1
Berkeley African feldspar B		\ - '	` , ,		
run 1	4.49	(¶)	0.1054 (1,63 Myr BP)	(D)	1
run 2]		\ • /	0.0941 (1.46 Myr BP)	Õ	1
Berkeley African feldspar C	5.74	(¶)	0.1531 (1.85 Myr BP)	ďĎ	1

Personal communications from †J C Engles, ‡M. Lanphere, § E. Jager.

Two samples of BKT-2u were collected independently, from the Bouroukie wadi, a tributary to the Kada Hadar drainage (Fig. 3). Each sample was gently wet-disaggregated to preserve the original grain sizes. The samples were washed for 10 min in dilute (\sim 4%) HF, and sonified to rid the minerals of adhering clay particles, thoroughly rinsed with de-ionized H₂O and air dried. They were subjected to density, sieving, and magnetic separations.

BKT-2u contains a simple, euhedral, volcanic phenocryst assemblage consisting of 25% anorthoclase, 1% aegerine-augite, and common magnetite octahedra and zircon. The phenocrysts are encased in a matrix of collapsed pumice clasts. Small, 0.05–0.10 mm, magnetite octahedra and zircon crystals are common inclusions in the larger, 0.50–1.50 mm anorthoclase and aegerine-augite phenocrysts⁵. In addition, BKT-2u contains 0.5–2.0 mm, angular fragments of vesicular basaltic glass previously misidentified as obsidian, and basaltic rock fragments, perhaps incorporated during a mixed magma eruption¹. Anorthoclase and zircon were used for K/Ar and fission track dating, respectively^{1,4–6}.

One of the collected BKT-2u samples, sample 1, was subjected to a very refined size, density and magnetic separation to isolate anorthoclase of different physical properties that could be tested for K/Ar age concordance.

The first K/Ar age reported for BKT-2u was 2.63 ± 0.05 Myr BP (ref. 5), which is the mean and 1σ standard deviation of four measurements of the same light, non-magnetic, 35/80 mesh anorthoclase fraction from sample 1(tracers 277, 279, 297, and 311; Table 2). Additional measurements were reported on two finer size fractions of sample 1 anorthoclase (tracers 470 and 471; Table 2) and on a dense, magnetic separate in which each anorthoclase crystal contains a small (\sim 0.5 mm) octahedral magnetite inclusion (tracer 472; Table 2). We also report two new K/Ar measurements made on the second BKT-2u sample, sample 2 (tracers 874 and 876; Table 2) and three measurements for a 35/80 mesh anorthoclase fraction separated from BKT-2L (tracers 466, 467 and 468; Table 2). Note that sample 2, BKT-2u, was not subjected to such a refined density and magnetic separation as sample 1.

In all, 14 conventional K/Ar measurements have been made on BKT-2. These are listed in Table 2, corrected for new decay, abundance and tracer constants. The 14 age determinations yield a mean age and standard deviation of the measurements of 2.93 ± 0.11 Myr BP with an absolute range 2.75-3.12 Myr.

Note, however, that the K/Ar measurements of sample 2, BKT-2u, yield a slightly older set of ages, from 3.02 to 3.12 Myr. This compares with the range of sample 1 measurements from 2.75 to 2.98 Myr. The mean and standard deviation for sample 2 and sample 1 are 3.07 ± 0.05 and 2.88 ± 0.08 Myr BP, respectively. Zircon fission track data show that BKT-2u contains two zircon age populations; a dominant one around 2.7 Myr BP and a minor component around 5.0 Myr BP (ref. 1). This suggests that the older K/Ar measurements on sample 2 could be due to trace amounts of possible contaminating feldspars associated with the older zircon component. The younger set of K/Ar ages

measured for sample 1 could be due to the exclusion of detrital feldspars because of the more refined laboratory separation process that sample 1 underwent. However, even sample 1 feldspars may have suffered some contamination.

The concordance of all K/Ar measurements for various fractions of sample 1 argues that any contaminating effect is minimal. The concordant age group, 2.88 ± 0.08 Myr BP, includes measurement of the magnetic anorthoclase fraction in which every feldspar grain contains an octahedral magnetite inclusion. We feel that this inclusion trait fingerprints the BKT-2 U and L eruptions, and is too rare for the tephra to contain contaminants of similar grains from an older eruption. Finally, we stress that the lever arm exerted on the measured age by detrital contamination is much less at Hadar than at most East African sites. This is because the sediments at Hadar are dominantly volcaniclastic with measured K/Ar and fission track ages on the detrital components being less than 30 Myr (ref. 6).

We accept the 2.88 ± 0.08 Myr BP mean age as the most precise K/Ar measurement of the BKT-2 eruption. The fission track age of the dominant younger component of BKT-2u is 2.7 ± 0.2 Myr BP (ref. 1), which is within the uncertainty of the K/Ar age but further work is needed to improve its precision.

Kadada Moumou basalt

The Kadada Moumou basalt (KMB) crops out along the Ounda Hadar wadi (Fig. 3), in the upper part of the Sidi Hakoma (SH) Member of the Hadar Formation (Fig. 1). The correlation of KMB with the fossil hominid localities in the lower SH Member, such as A.L. 200, is not precisely established. The SH Member

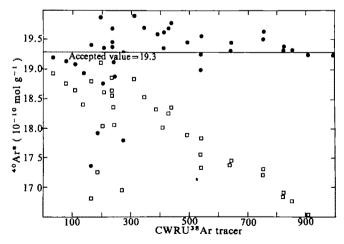


Fig. 2 Monitoring the depletion of the CWRU (Case Western Reserve University) ³⁸Ar tracer bulb against the LP6 biotite interlaboratory standard. Method A (□) utilizes glassblower-stipulated ³⁸Ar in the initial fill and an 18.6×10⁻¹⁰ mol g⁻¹ of ⁴⁰Ar* value for LP6. Method B (●) uses the machinist stipulation of the pipette volume and 19.3×10⁻¹⁰ mol g⁻¹ of ⁴⁰Ar* for LP6. Method A is wrong, method B is correct.

Sample fused on previously degassed young basalt

Accepted values have not been published.

[#] Typical weight 0.15 g.

Table 2 Conventional K/Ar data for BKT-2 feldspars

Sample				#	
•	weight				
Sample no, fraction, tracer run no	weight (g)	% K₂O	40 Ar* (×10 ⁻¹⁰ mol g ⁻¹)†	40Ar*/40Ar _T	Age (Myr)‡
	æ,	/0 IL ₂ O	n (Alo morg)	2 MA / 2 MAT	1480 (14171)+
BKT-2u, sample 1					
Sink 5, non-magnetic, 35/80 mesh					
277 .	3.8000	1.96	0.0778	0.77	2.75 ± 0.08
279	5.3850	2.00	0.0814	0 85	2.82 ± 0.08
297	4.1901	1 96	0.0810	0 80	2.86 ± 0.08
317	4.3815	1.96	0.0829	0.67	2.94 ± 0.08
873	2.0007	1.90	0.0793	0.40	2.89 ± 0.08
Sink 5, non-magnetic, 100/140 mesh					
470	4.0000	1 83	0 0789	0 73	2.98 ± 0.08
Sink 5, non-magnetic, 80/100 mesh					_,,
471	4.0000	1.87	0.0795	0.68	2.94 ± 0.08
Sink 2, magnetic, 35/80 mesh§				****	
472	4,0000	1 73	0.0730	0 77	2.93 ± 0.08
BKT-2u, sample 2					
Sink 3, non-magnetic, 35/60 mesh					
874.	2.0004	1.77	0.0769	0.34	3.02 ± 0.08
Sink 3, non-magnetic, -170 mesh					
876 Step 1 argon extraction	2 0001	1 85	0 0823 .	0.39	3.09 ± 0.08
Step 1 + higher temperature step		****	0.0823	0.39	3.09 ± 0.08
BKT-2L, Sink 4, non-magnetic, 35/80 mesh					
466	4.0000	1.47	0.0624	0.62	2.95 ± 0.08
467	4 0000	1.47	0.0585	0.68	2.76 ± 0.08
468	4.0000	1.47	0 0603	0.60	2.85±0.08
400	4.0000	2,77	0 0000		$d_1 = 2.92 \pm 0.11$
•		•	•	Wican a	u, 2,72 = 0,11

thickens from ~70 m at Kada Hadar, where no basalt occurs but where numerous hominid localities exist, to ~160 m at Ounda Hadar and Ourda, where the basalt crops out, but where no hominids are found (Figs 1 and 3). KMB is probably stratigraphically above the lower SH Member hominid finds, as judged by (1) proportioning the stratigraphical thicknesses between the Kada Hadar and Ounda Hadar-Ourda sections: (2) by tentative correlation of gastropod-bearing layers above most of the hominid levels with similar layers below the basalt; and (3) by correlation of a prominent palaeomagnetic polarity transition, from normal to reverse, that occurs below the basalt in the Ounda Hadar section and above the gastropod layers in both the Ounda Hadar and Kada Hadar sections (Fig. 1)5. The KMB is, however, stratigraphically below such important hominid localities as A.L. 333 and A.L. 288 in the Denen Dora (DD) and Kada Hadar (KH) Members, respectively, because the KMB is definitely below the Triple Tuff (TT) and Ostracod clay marker horizons which form the base of the DD Member (Fig. 1).

Initial chronological measurements for the Hadar Formation centred around the KMB^{4,5}. This lava is a moderately low

Table 3 Whole rock chemical data* for the Kadada Moumou basalt Type A-1 (n = 3)A-2 (n = 4)B(n=3) S_1O_2 51.16 52 01 52.13 Al₂Ō₃ 13.89 13 77 13.73 8 47 FeO 8.80 9.71 4.20 3.41 Fe₂O₄ 4 16 2.90 TiŌ₂ 2-94 ΜnŌ 0.23 0 24 MgO 4.23 3.97 4 36 7 87 774 CaO 8 47 Na₂O 3 58 3 67 3.57 K₂Ō 074 0 70 0.69 0 66 0.62 LOJ. 0 75 0.92 0.49 Total 99.52 99.76 99.28 Rb 23 23 22 Sr 206 303 298 Nı 40 40 43 Zr 365 376 392 Ba 312 317 285 0.077 0 076 0.074 280 285 270 K/Rb

potassium (~0.6% K₂O) tholeiite, which displays various stages of alteration depending on position in the 2-3 m thick flow. The base of the flow is conformable with sediments of the Hadar Formation. The sediments were baked to a depth of 2-3 cm, and the base of the flow was supercooled forming a 2-3 cm thick chilled zone. Protruding upwards from this contact are abundant bent 15-cm long calcite-filled pipe vesicles. These contact features suggest that the lava erupted over very shallow water or over water saturated sediments. The sedimentary environment over which the lava flowed is interpreted as a floodplain or marsh⁸. The reaction between lava and water-saturated sediments probably caused severe hydration of the lava¹.

Type A and B Kadada Moumou basalts

Recovery and dating of a new, unique basalt sample from the Kadada Moumou flow is a major factor which caused us to revise our minimum age estimate of 3.0 ± 0.2 (refs 4, 5) to $3.60\pm$ 0.15 Myr (ref. 1). The unique variety of this flow is termed 'type B'; all previously published data for KMB were accomplished on 'type A' varieties. These two varieties can be distinguished petrographically as well as geochronologically.

In general, the KMB is not ideal for dating because of variable alteration of the basaltic glass and mesostasis to a nontronite clay. The alteration presumably developed diagenetically after initial hydration of the flow, as described above. Being aware of the variable amount of clay alteration in KMB⁵, we conducted a detailed search of the flow and recovered a uniquely leastaltered sample, KM76-5. This sample was collected in situ from the core of an oblate, spherical structure about 20 cm in diameter, within the middle of the 3-m thick flow. The core of the sample consists of dense, fine-grained basalt that grades radially outward to a zone containing minor flattened, nontronite-filled amygdules. The rim of the structure contains abundant, flattened, and rounded nontronite-lined and filled vesicles. This structure conceivably originated as a cooled block of the lava flow reincorporated into the flow. Its major and trace element chemistry is identical to the rest of the flow (Table 3). The petrography of type B KM76-5 is, however, unique in that it is more nearly holocrystalline (Table 4) and essentially all of its glass is unaltered. In addition, the plagioclase and pyroxene of the type B variety are finer grained than type A varieties (Table 4) and the magnetite is equant (cubic) rather than rod-shaped

[†] Data corrected for new ³⁸Ar depletion constant ‡ Data corrected for new ⁴⁰K decay and abundance constants.

[§] Each grain contains a magnetite octahedron

^{*} X-ray fluorescence

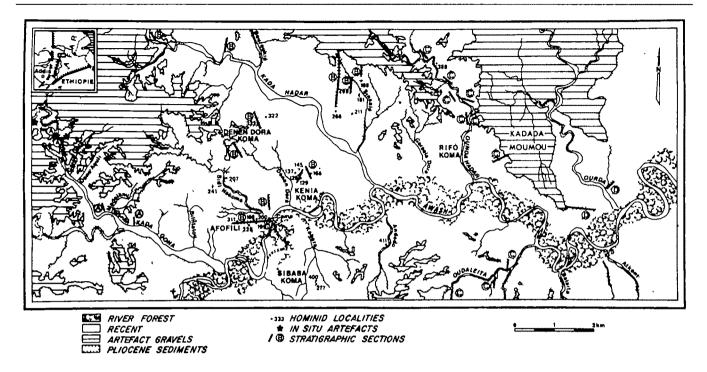


Fig. 3 Map of the Hadar hominid site showing location of stratigraphical sections and hominid localities (taken from ref. 8).

(acicular). Type A samples contain significantly greater amounts of glass, altered glass, and nontronite than type B samples, and their crystals are much coarser grained (Table 4).

Initially, a random piece of KM76-5 was prepared for K/Ar analysis. Subsequently, two additional samples were obtained by sectioning the core of the boulder into thin slabs. The sample was then sawn into two fractions, one labelled F (fair), which contained minor nontronite-filled vesicles, and the other labelled G (good), which was less vesicular. The nontronite in the type B basalt is thought to be a secondary amygdale fill and not an alteration product of the basaltic glass because, in thin section, the nontronite can be seen to be relegated to vesicles, and virtually none of the matrix is altered.

K/Ar data

The resultant mean and standard deviation of eight measurements of the KM76-5F sub-sample is 3.64 ± 0.12 Myr BP. For five measurements of the KM76-5G sub-sample this is 3.54 ± 0.12 Myr BP. Three runs of the original bulk sample, KM76-5, yield a mean value of 3.67 ± 0.10 Myr BP. Thus, the 16 measurements of the three sub-samples of the type B variety are internally concordant with a mean age of 3.61 ± 0.13 Myr BP.

In addition, type B samples produced uniformly higher proportions of radiogenic argon, ~25%, compared with only

~15% radiogenic argon for type A samples (Table 5). This enhancement of radiogenic argon is mainly due to a reduction in contaminating atmospheric argon, the additional radiogenic argon being a minor component. Typically, we and others observe the general correlation that the per cent radiogenic argon yield is inversely proportional to the amount of secondary alteration 1,10-12. We interpret the lack of alteration of the type B sample as due to the dense core structure preventing access of altering solutions.

In contrast to the type B variety of the flow, the type A basalts produce uniformly lower measured ages (Table 5, revised for new constants and tracer factor) with some ages as young as 3.0 Myr (revised; petrographical type A-1) and others higher at 3.3 Myr (revised; type A-2). K₂O and Ar measurements on physical separates of the sugary-textured2 type A-1 basalt were attempted to see if a single sample could be shown to be internally discordant between low density glass-rich, nontronite-rich fractions and heavy glass-poor, nontronite-poor fractions. Initially, a rough separate into two density fractions was performed on type A-1 sample KM-2-74 (lab) and the heavier fraction was analysed (sample 1, Table 6). A large separate piece (sample 2) was then disk pulverized and subjected to a careful size/density separation into eight fractions (sample 2, Table 6). Modal counts were made on grain mount

Table 4 Modal mineralogy of type A and type B Kadada Moumou basalts and type A density separates KM2-74 (lab) (type A-1) Grain mounts Sink 4 A-2 Sink 1 Sink 2 Sink 3 Sink 4 Sink 2 Sink 3 Type and no. analysed (n=3)(n=3)(n=3)100/120 100/120 100/120 100/120 80/100 80/100 80/100 80/100 Mode* Plagioclase* 10 24 Chnopyroxene* Opaque* 13 3 50 20 0 12 10 2 6 7 Plagioclaset 18 29 12 16 Pyroxenet 11 33 11 15 12 Opaquet Glassi 17 32 16 31 11 45 20 30 41 Alteration†‡ 15 10 Vesicles † 2 1 Calcutet

Mode is based on 1,000 counts. * Phenocryst phases (>0.05 mm) † Groundmass phases (<0.05 mm), t, trace ‡ Includes vug and intersertal fill

Table 5 Conventional K/Ar data for the Kadada Moumou basalt						
Sample and tracer no	Type '	Sample weight (g)	% K₂O	40Ar* (×10 ⁻¹² mol g ⁻¹)	.40Ar*/40Ar _T	Age (Myr)‡
KM-2-74 (lab)	A-1 1	10.0001	0.764	=	0.14	2.00 . 0.05
258 860		10.0001	0.764	3 405	0 14	3.09 ± 0.25
	A- 1	7.9988	_	3.345	0.14	3.08 ± 0.26
KM-3-74		,				
257	A-1	10.0001	0 747	3.110	0 26	2.88 ± 0.20
262†	A-1	10.0000		3.210	0.15	2.98 ± 0.25
263	A-1	10.0000		4.112	0.24	3.80 ± 0.22
865	A-1	8.0041		3,252	0.18	3.02 ± 0.20
KM75-123-3	,			i		
339	-A-1	7.9992	0.673	2 633	0 16	2.71 ± 0.25
360	A-1	7.3755		2 826	0 19	2.86 ± 0.25
840	A-1 :.	7 0004		2.910	0.15	2.92 ± 0.25
KM-1-73					,	
261	A-2	9.9997	0.657	3.007	0.17	3.21 ± 0.23
•		7.3331	0.057	5.007	0.17	3.21 2 0 23
KM-1-74 (lab)	1					
259	A-2	10.0001	0.727	3.538	0.15	3.38 ± 0.24
859	A-2	10.0000		3.534	0.15	3.38 ± 0.24
KM4-74			•			
256	A-2	10.0001	0.686	3.237	0 14	3.28 ± 0.23
260	.A-2	10.0001		3.269	0.13	3.38 ± 0.26
866	A-2 '	8 0039		3.292	0.12	3.36 ± 0.28
KM76-5					•	
609	В	7.0017	0.656	3.484	0.21	3.68 ± 0.25
683	B	7.0041	0.050	3 576	0.21	3.78±0.25
787	B +	7.0051		3 358	0.18	3.55 ± 0.25
	,				**-*	5,500 5,20
KM-76-5G	70	7.0000	0.687	2.165	0.10	224.024
658	B B	7 0009	0.657	3.165	0.19	3.34±0 24
786		7 0027		3.349	0.19	3.54 ± 0.24
812	В	7.0063		3 507 3.359	0 24	3.70 ± 0.22
867 868	B '	10 0001 10.0003		3.363	0.21 0.21	3.56 ± 0.22 3.56 ± 0.22
	ъ .	10.0003		3.303	0.21	3.30±0.22
KM76-5F				*		
659	В.	7.0093	0.659	3.416	0.25	3.59 ± 0.22
716	В	7.0035		3.452	0 27	378 ± 022
813	В	6.9999		3.460	0.27	3.65 ± 0.21
835	B .	9.9939		3 459	0.29	3.65 ± 0.20
841	В	7.0045		3.418	0.27	3.61 ± 0.22
861	В ,	9.9999		3.187	0.31	3.36 ± 0.20
869	В	10.0051		3.540	0.23	3.72 ± 0.22
870	В	10 0003		3.609	0 25	3.80 ± 0.21

† Not baked out.

‡ New constants and tracer depletion factor.

Table 6 Szze/density fractionation experiment KM2-74 (lab) type A-1						
Sample and fraction	³⁸ Ar tracer no	Weight (g)	K₂O %	⁴⁰ Ar* (×10 ⁻¹² mol g ⁻¹)	⁴⁰ Ar*/ ⁴⁰ Ar _T	Age (Myr)
Sample 1	ŧ					
12/35 mesh, raw, run 1	258	10.0	0.76	3.40	0.153	3.09 ± 0.20
12/35 mesh, raw, run 2	860	8.0	0 76	3 34	0.140	3.08 ± 0.20
80/100 mesh gross heavy fraction	899	8 1	0.73	3.85	0 146	3.63 ± 0.28
Sample 2 80/100 mesh					•	
Raw, unfractionated	1,078†	8.5	0 787	3.81	0.130	3.36 ± 0.27
Sink 1 (heaviest)	1,073†	9.0	0 525	2 53	0.116	3.33 ± 0.31
Sink 1, repeat run	1,092	10.1		2.69	0.141	3.55 ± 0.32
Sink 2	1,074†	9.0	0 971	4.33	0.131	3.09 ± 0.26
Sink 3+4 combined	1,075†	11.0	1.139	4 95	0 135	3.02 ± 0.28
100/120 mesh				•		
Sink 1	1,076†	· 5.5	0 413	2.13	0.101	3.57 ± 0.41
Sink 2 (no Ar run)	´ - '		0.807	_		
Sink 3	1,077†	7.0	1.073	4.82	0.138	3.13 ± 0.28
Sink 4 (no Ar run)			1.122	_		

† Unbaked.

thin sections (Table 4) and 6 fractions were analysed for K_2O and Ar (Table 6).

The heavier fractions, being poor in glass and nontronite and rich in pyroxene (Table 4), are considerably lower in K_2O (0.4–0.5% K_2O). The light fractions are rich in glass, nontronite, and plagioclase (Table 4) and high in K_2O (1.07–1.12% K_2O ; Table 6). The heaviest fractions (sink 1) produce uniformly older ages, with four measurements having an average and standard deviation of 3.5 ± 0.5 Myr. For this separation experiment most of the separates were not pre-baked and yielded a low percen-

tage of radiogenic argon that resulted in a large internal imprecision in the individual argon measurements.

These results, though imprecise, indicate that the altered type A basalt is internally discordant. The data associate younger ages with more abundant glass, a phase that is prone to argon loss, or with more nontronite that is, in part, observed to be an alteration product of glass in type A basalts.

The possibility that the type B Kadada Moumou basalt has some excess magmatic ⁴⁰Ar has to be considered. We think this is unlikely, however, because of the rarity of examples of excess

argon in aphanitic terrestrial basalts¹⁵. The flow is extensive and thin enough that it should have thoroughly degassed. Furthermore, there is no evidence for older acidic crust in western Afar¹ that could have contaminated the magmas as was indicated for the New Zealand basalts studied by McDougall et al. 13; no xenoliths of cognate inclusions have ever been found in either the Kadada Moumou flow or numerous other western Afar flows¹. It is also unlikely that the flow picked up boulders or fragments of older basalt because the flow erupted onto overbank sediments of clay and clay-sized particles. On the contrary, there is a distinct inverse correlation of whole rock measured ages with degree of observed sample alteration and, in the type A fractionation experiment, with glass and nontronite content (Tables 4-6). Alteration typically causes ⁴⁰Ar loss (and K gain) in K/Ar systems. Therefore, we regard the spectrum of ages obtained from the Kadada Moumou flow as being due to alteration variably affecting the K/Ar age measurement. We regard the 3.60 ± 0.15 Myr BP age measurement of the type B basalt, the least altered variety of the flow, as a best-estimate minimum age for the flow. However, even the type B age requires some qualification because of the small, but perhaps significant, amount of alteration also seen in this variety.

Accepting the older 3.60 Myr age for the Kadada Moumou basalt necessitates a revised correlation of the Hadar palaeomagnetic sequence to the palaeomagnetic time scale: the details will be considered elsewhere. The Kadada Moumou flow and

Received 13 July, accepted 29 December 1981

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overlying and underlying sediment are reversed. The revised age places the reverse sequence within the Gilbert Epoch which ended 3.4 Myr BP instead of in the Mammouth Event 3.15-3.05 Myr BP as previously interpreted (Fig. 1).

Conclusion

Adoption of new decay and abundance constants, recalibration of the ³⁸Ar laboratory tracer pipette and data from several new samples requires us to revise the K/Ar ages for the BKT-2 tephra, from 2.65 to 2.9 Myr BP, and the Kadada Moumou basalt, from 3.0 to 3.6 Myr BP. All the fossil hominid remains at Hadar are found below BKT-2, and many hominid localities occur stratigraphically below the Kadada Moumou basalt. Therefore, on the basis of this revised chronology, the fossil hominid material in the Hadar Formation is interpreted to range from at least 2.9 Myr BP to somewhat older than 3.6 Myr BP. These revised ages permit a correlation of the pre-basalt Sidi Hakoma Member hominid fossils at Hadar with the footprints and fossils at Laetoli which is 700 km to the south and well dated between 3.6 and 3.75 Myr BP (new constants)¹⁶

This work was supported by NSF. We thank S. A. Mertzman and Franklin and Marshall College for use of XRF facilities, and Derek York for reading the manuscript. Rick Cotman, Bob Sedivy and Larry Dasch helped with the K/Ar and mineral separation laboratory. We thank Karen Toil for helping to prepare this manuscript.

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Initiation of ice ages by creep instability and surging of the East Antarctic ice sheet

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Creep or shear heating instability is shown to be a plausible means of initiating a surge of the East Antarctic ice sheet and plunging the Earth into an ice age. The occurrence of this instability requires only that the ice thickness exceed a critical value which may not be much larger than the present ice dome thickness. Creep instability can occur on the fast time scale of 100-1,000 yr required by inferences of past rapid cooling events and sudden rises of sea level before the onset of Northern Hemisphere glaciations.

THE Antarctic surge theory for the inception of ice ages invokes an instability in the ice sheet to melt its base and cause it to slide into the ocean, thereby forming a huge ice shelf around the southern continent. This would increase the Earth's albedo and lead to global climatic cooling and the inception of glaciation in the Northern Hemisphere. It would also produce a rapid rise in sea level by as much as 15-20 m at the end of an interglacial². Several lines of evidence indicate that these events did indeed occur near the end of the last interglacial3-6; in particular, the association of the >8 m sea level rise ~120,000 yr ago inferred from raised coral reefs in New Guinea with the 2 °C cooling of the surrounding ocean on a 1,000 yr time scale deduced from oxygen isotope studies of giant clam shells strongly supports the surge theory6. The magnitude of the sea level rise requires the surge of a large fraction of the East Antarctic ice sheet^{5,7}; disintegration of the West Antarctic ice sheet would increase sea level by only ~4 m (refs 8, 9).

The only physical mechanism which seems capable of initiating a surge of the East Antarctic ice dome, or major portions thereof, is creep instability due to frictional heating at the base of a deforming ice sheet 10-16. Such an instability could cause massive melting at the base of the ice sheet on a very rapid time scale and allow the ice to slide freely over the underlying bedrock. The possibility of melting by shear heating is a consequence of the strong temperature T dependence of the rheology of ice. Frictional heating in the basal shear layer of the ice sheet tends to raise the basal temperature and reduce the viscosity near the base. If the thickness of the ice sheet remains constant, so will the basal shear stress, and the increase in temperature and decrease in viscosity will tend to increase

the rate of frictional heating. Enhanced heating will further increase the temperature in a potentially unstable manner. Shear heating instability can occur if the ice sheet thickness h exceeds a critical value h. in which case steady, subsolidus sliding of the ice sheet is not possible 15

The scenario we develop calls on climatically enhanced accumulation to increase the thickness of the East Antarctic ice sheet above the critical value necessary for the onset of explosive creep instability. The climatic change could be a consequence of latitude-dependent variations in solar insolation that accompany changes in the Earth's orbital motion. Thus our model may provide a link between the astronomical 17,18 and Antarctic surge theories for the inception of ice ages by using the former to initiate the latter. However, if the Antarctic surge phenomenon does have a crucial role then it is not necessary for orbital forcing to cause major spreading of ice sheets. Any climatic occurrence resulting in increased accumulation over Antarctica and a sufficiently large thickening of the Antarctic ice sheet might initiate surging and the onset of an ice age.

Model

The critical thickness h_c required for creep instability and surging can be calculated using a one-dimensional, steady-state, thermomechanical model for the gravitational creep of a constant thickness h ice sheet down a slope making an angle α to the horizontal¹⁵. The coordinate perpendicular to the slope is y; the ice surface is y = 0 and the base is y = h. The surface of the ice and its base have the same slope in this model, while the surface and bedrock slopes of the East Antarctic ice sheet are unequal. This difference between the mathematical model and the real ice sheet is unimportant, however, as the shear stress τ in an ice sheet is mainly determined by the surface slope¹⁹. The distribution of shear stress in our model ice sheet,

$$\tau = -\rho g y \sin \alpha \tag{1}$$

where ρ is the density of ice and g is the acceleration due to gravity, is thus representative of the shear stress in the real ice sheet as long as we equate α with the local surface slope.

The equations governing the temperature T in the model ice sheet and its downslope creep velocity u are 15

$$\frac{\mathrm{d}^2 T}{\mathrm{d}y^2} - \frac{v}{\kappa} \frac{\mathrm{d}T}{\mathrm{d}y} + \frac{2A}{k} \{ \rho gy \sin \alpha \}^4 \exp \left(\frac{-E^*}{RT} \right) = 0 \tag{2}$$

$$\frac{\mathrm{d}u}{\mathrm{d}v} = -2A \{\rho g y \sin \alpha\}^3 \exp\left(\frac{-E^*}{RT}\right) \tag{3}$$

where k is the thermal conductivity and κ is the thermal diffusivity. The temperature equation (2) is a balance between conduction, vertical advection with velocity v, and viscous dissipation. Equation (3) is the creep law of ice²⁰, A is a rheological , A is a rheological parameter, E^* is the activation energy and R is the universal gas constant. The vertical velocity is given by the accumulation rate. We use two models for the y-dependence of $v: v = v_c =$ constant and $v = v_c(1 - y/h)$. The constant v model is preferred because v decreases from v_c to 0 in a thin boundary layer at the base of the ice sheet¹⁵. Equations (2) and (3) are solved subject to the conditions

$$T = T_0, \qquad \text{on } y = 0. \tag{4}$$

$$T = T_0$$
, on $y = 0$. (4)
 $k \frac{dT}{dy} = q_b$, $u = 0$, on $y = h$ (5)

where T_0 is the constant temperature of the ice surface and q_b is the geothermal heat flux entering the base of the ice sheet. We have assumed that there is no basal sliding. This two-point boundary value problem is solved using standard numerical procedures¹⁵.

Parameter values

The East Antarctic ice sheet has surface slopes of 0.05-0.10° and thicknesses of 2-4 km (refs 21-24); accumulation rates are typically 0.03 m yr⁻¹ in the interior and an order of magnitude larger near the coast^{14,25}. Experimental data on the creep of polycrystalline ice suggest that E^* is in the range 60-70 kJ mol⁻¹ at temperatures ≤ -10 °C (refs 26–28). The value of A depends on grain size, impurity content and crystal orientation. We have used $A=8.75\times10^{-13}\,\mathrm{s^{-1}\,Pa^{-3}}$, a value representative of the experimental determinations^{27,28}. We have also used $k=2.5\,\mathrm{W\,m^{-1}\,K^{-1}}$, $\kappa=1.3\,\mathrm{mm^2\,s^{-1}}$, $\rho=900\,\mathrm{kg\,m^{-3}}$, $g=9.8\,\mathrm{m\,s^{-2}}$, $T_0=223\,\mathrm{K}$, and $q_b=40\,\mathrm{mW\,m^{-2}}$. The value of T_0 is representative. tive of the annually averaged surface temperature in the interior of East Antarctica and the value of q_b is a typical continental heat flux29.

Critical thickness for shear heating instability

Nonlinear coupling between velocity and temperature results in the solution curves shown in Fig. 1 for $E^* = 60 \text{ kJ mol}^{-1}$. For given α and v_c there is a maximum thickness h_c for which steady creep is possible. The dashed portions of the curves indicate solutions which have basal temperatures at or above the melting temperature of ice. The maximum thickness increases with increasing accumulation rate (Fig. 1a) because downward advection of cold ice to the basal shear zone enhances the

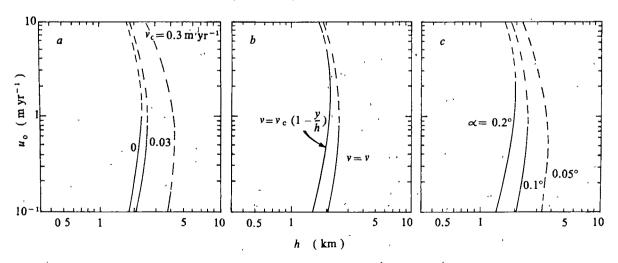


Fig. 1 Surface velocity u_0 as a function of ice sheet thickness for $E^* = 60 \text{ kJ mol}^{-1}$. a, Effect of varying the accumulation rate, $\alpha = 0.1^\circ$, $v = v_0$; b, influence of vertical velocity profile, $\alpha = 0.1^\circ$, $v_c = 0.03 \text{ m yr}^{-1}$; c, effect of varying surface slope, $v_c = 0.03 \text{ m yr}^{-1}$, $v = v_c$. For given α and v_c there is a maximum thickness for which stable, steady, gravitational sliding is possible.

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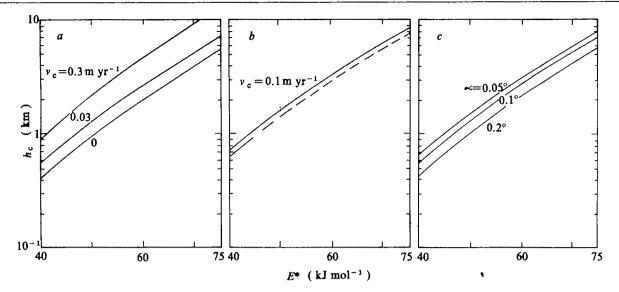


Fig. 2 Maximum thickness h_c for stable, steady, downhill creep of an ice sheet as a function of activation energy E^* . a, Effect of varying the accumulation rate, $\alpha = 0.1^\circ$, $v = v_c$; b, influence of vertical velocity profile, $\alpha = 0.1^\circ$, $v = v_c$ (solid), $v = v_c(1 - y/h)$; c, effect of varying surface slope, $v_c = 0.03$ m yr⁻¹, $v = v_c$.

importance of frictional heating. For the same reason, h_c in the constant v model is larger than it is in the situation where v decreases linearly with depth (Fig. 1b). A decrease in α also leads to an increase in h_c (Fig. 1c) because large ice thicknesses are then necessary to produce the shear stresses required for internal deformation. Basal temperatures for the supercritical states are generally at or above the melting point of ice. Even the subcritical states have basal melting when the ice thickness is large. The tendency of downward advection to lower basal temperatures is more than compensated by the associated increase in viscous dissipation.

Figure 2 shows the dependence of the maximum thickness on activation energy; h_o increases approximately exponentially with E^* for given α and v_c . The thicknesses within the East Antarctic ice sheet (2–4 km) are near the calculated values of h_o for $\alpha=0.1^\circ$, $v_o=0.03$ m yr⁻¹ and E^* between 60 and 70 kJ mol⁻¹.

Occurrence of instability and surging

The existence of a maximum thickness for which steady, stable, subsolidus, gravitational sliding of an ice sheet is possible is the fundamental reason for the occurrence of catastrophic shear heating instability. According to Fig. 2, for example, an ice sheet on a 0.1° slope would creep down hill in a steady, stable manner if its thickness were ≤5 km for an activation energy of 70 kJ mol⁻¹ and an accumulation rate of 0.03 m yr⁻¹. However, if its thickness were suddenly increased above 5 km by a rapid climatically enhanced accumulation it could no longer slide stably. An instability would occur in which frictional heating near the base of the ice would result in massive melting and a surge of the ice sheet, as shown in Fig. 3. The maximum thickness of the East Antarctic ice sheet is ~4 km, its activation energy may be $\sim 70 \text{ kJ mol}^{-1}$, surface slopes of about 0.1° occur over widespread areas, and typical accumulation rates are 0.03 m yr⁻¹. Thus it is possible that the East Antarctic ice sheet lies close to the point of creep instability.

The occurrence of shear heating instability and surging in response to a rapid increase in ice thickness $h > h_{\rm c}$ is a consequence of the super-exponential time scale on which thermal runaways of this nature take place³⁰. The growth times for such explosive finite-amplitude instabilities far exceed those calculated on the basis of linear theory^{31,32}. The very rapid growth of the instability limits the time available for the ice sheet to self adjust to the increase in accumulation in ways that might avoid instability. For example, if the flux could be maintained constant by thinning the ice sheet, it would be stabilized, because the solutions for flows subjected to a constant flux

boundary condition are single-valued in contrast to the multivalued solutions occurring for the constant thickness boundary condition^{33,34}. However, the sudden enhancement in accumulation is externally imposed, the ice sheet must thicken initially, and the catastrophic occurrence of shear heating instability precludes self adjustment and the maintenance of constant flux^{35,36}. Simple energy arguments support the reality of the instability³⁷.

The fast time scale associated with finite-amplitude shear heating instability is essential to ice sheet surging on a continental scale; the isotopic evidence⁶ for rapid surging at the end of the last interglacial has already been cited. It is probably not feasible for basal melting in subcritical conditions to lead to large-scale disintegration of the Antarctic ice sheet, as originally suggested by Wilson¹, because there is no fast time scale involved. The ice sheet has sufficient time to flow and accommodate itself to changing surface conditions so as to preclude massive collapse by large-scale decoupling between the ice and the bedrock. This is supported by inferences from modelling^{38,39} and the discoveries of large sub-ice lakes23 that large areas of the base of the East Antarctic ice sheet are at the melting temperature. Despite this, the ice sheet is apparently stable. This does not preclude localized basal sliding or surging due to melting by shear heating. Basal sliding at the rate of 10 m yr⁻¹ has been reported for the ice sheet in the Mizuko Plateau⁴⁰.

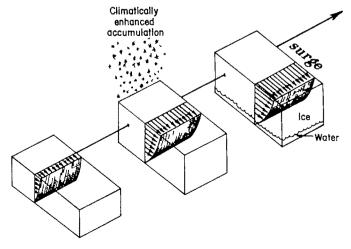


Fig. 3 Sketch of the events leading to shear heating instability and surging of the East Antarctic ice sheet.

for example. Such localized sliding contributes to the global stabilization of the present ice sheet.

The above modelling results suggest that past ice ages may have been caused by shear heating instability of the East Antarctic ice sheet. A climatically enhanced accumulation is required to thicken the ice beyond its maximum thickness for stable, steady, gravitational sliding. Any climatic event which enhances accumulation over Antarctica would suffice to initiate the process; variations in the Earth's orbital motion or enhanced volcanic activity are possibilities. The thickening of the ice must occur quickly so that flow within the ice cannot lead to an

Received 30 November 1981, accepted 20 January 1982.

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accommodation and stabilization of the ice sheet against the changing conditions. Instability and large-scale melting must occur extremely fast for the same reasons. Because shear heating instability is explosive in nature, such fast collapse of the ice sheet should be possible. Theoretical calculations of the super-exponential growth rates accompanying these catastrophic instabilities yield the necessary fast time scales.

D.A.Y. thanks the NATO Postdoctoral Fellowship Program, NSERC of Canada, and the Research Corporation for financial support. This research was also supported by NSF grant DPP80-

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Is the Sun an oblique magnetic rotator?

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The recent observation of rotational splitting of the non-radial p modes of l=1 and l=2 (with $n\sim 20$) of the 5-min global oscillation of the Sun was interpreted in terms of rotational splitting associated with a rapid rotation of the solar interior. The precise value deduced for the angular velocity Ω of the interior depends on the assumed variation of Ω with depth and on the weighting function. Thus with a weighting function² of the form

$$\bar{\Omega} = \int \frac{\Omega(r)}{V(r)} dr \int \frac{dr}{V(r)}$$
 (1)

where V(r) is the local velocity of sound at radius r, one obtains values of $\Omega_{\rm core}/\Omega_{\rm surface}$ ranging from 2 to 9 as the radius at which Ω , assumed to be constant for smaller r, ranges from 0.6 to 0.15 of the solar radius. Such a weighting function proportional to the time the wave spends at any particular radius seems very plausible for the effect of any parameter, be it angular velocity or magnetic field, on the propagation of nearly plane waves as they bounce between the centre and the surface. Here I attempt to correlate the size of the observed rotational splitting with the enigmatic 12.2-day variation in the measurement of solar oblateness discovered by Dicke³ previously⁴ and to interpret the observed near equality of the amplitudes of the m-components of the l=1 and l=2 rotationally split modes. This leads to the first empirical evidence that an asymmetric magnetic rotator with megagauss magnetic fields exists in the interior of the Sun. It also suggests that the magnetic energy of young stars is a sizeable fraction of their gravitational energy.

I speculate, together with Dicke³, that this observed periodicity and the rotational period of the core of the Sun are identical.

This assumption allows one to refine the model of the solar interior by demanding that up to a radius r the Sun rotates uniformly, say, at the sidereal rate of 0.95 µHz (corresponding to 12.2-days) and beyond that radius at the surface rate of 0.46 µHz and that the integral (1) equals the observed rotational splitting of about 0.78 µHz (sidereal). On the above assumptions the integral can be satisfied for a current solar model' with the boundary at $r = 0.9 R_{\odot}$. It is natural to speculate that this value corresponding to the boundary between the rapidly rotating core and the slowly rotating surface layers does measure the depth of the convection zone. A slower transition from the rapidly rotating core to the more slowly rotating surface layers would imply a deeper convection zone.

The observation of l=1 triplets with nearly equal components (and less conclusively l=2 quintuplets with nearly equal components) does not accord with the expectation of purely rotational splitting of solar modes being viewed from Earth nearly along the solar equator. Thus, excluding the possibility of frequent excitation from randomly distributed sources and a correspondingly large damping, the expected skew symmetry of the central m=0 component about the solar equator would lead one to expect that the m=0 component ought to be absent in a global view of the Sun and very weak for a tilt of the solar equator of some 6° with respect to that condition as obtains during the observing period.

The presence of this component strongly suggests a perturbation which makes a large angle with the axis of rotation and rotates the angular distribution pattern through a substantial angle. Although purely hydrodynamic effects⁶ at the boundary between the rapidly rotating core and the slower outer layer could be responsible the required velocities would have to be high (~1 km s⁻¹) and in substantially different directions from those of the rotating matter. I, therefore, suggest that the perturbation is magnetic and in the absence of a theory of the combined action of rotation and magnetic field on frequencies of oscillations, that the analogy between rotational and magnetic splittings in an acoustic spectrum and magnetic and quadrupole splittings of atomic and nuclear energy levels be explored.

Such considerations suggest that an intense internal magnetic field, such as might be associated with an oblique (asymmetric?) magnetic rotator, could be responsible for the perturbation. Assuming a linear superposition of rotational and magnetic effects leads one to expect^{7,8} frequency shifts $\Delta \nu_{n,l,m}$ superimposed on acoustic frequencies ν of the order of

$$\left(\frac{\Delta \nu}{\nu}\right)_{n,l,m} = \alpha_{n,l,m} \frac{E_{\rm M}}{|E_{\rm G}|} \tag{2}$$

where $E_{\rm M}$ is the magnetic energy and $E_{\rm G}$ the gravitational energy of the Sun and $\alpha_{n,l,m}$ is of the order of unity and depends on the precise configuration of the magnetic fields and on the mode (n, l, m). The positions of the unshifted lines cannot be measured nor, at present, calculated. Upper limits on the magnetic energy can, nevertheless, be estimated through equation (2) from the symmetry of the observed multiplets together with the assumption that the obliqueness angle with respect to the rotational axis is near 90° (ref. 3). The l=1 triplets, observed between n=16 and n=26, are symmetric to within the measured linewidth of 0.6 µHz that is

$$\delta(\overline{\Delta\nu_{n,l}}) = \langle |\nu_{n,l,0} - \frac{1}{2}(\nu_{n,l,1} + \nu_{n,l,-1})| \rangle \le 0.6 \times 10^{-6} \text{ Hz}$$
 (3)

This implies that for $|E_{\rm G}|=6\times10^{48}\,{\rm erg}$ and $E_{\rm M}=(B^2/8\,\pi)\,\bar{V}$ where B is the magnetic field and \bar{V} is the volume of the Sun, excluding the convection zone, $\langle B^2\rangle_{\rm max}^{1/2}=3.3\times10^6\,{\rm G}$, if

$$\overline{\Delta \alpha} = \langle |\alpha_{n,l,0} - \frac{1}{2}(\alpha_{n,l,1} + \alpha_{n,l-1})| \rangle = 1 \tag{4}$$

whereas if $\Delta \alpha$ is substantially below unity the upper limit will be correspondingly higher.

A lower limit is given by demanding that the angular distribution rotates during the observation time $\tau = 28$ days by at least 1 rad that is $2\pi\Delta\nu\tau > 1$, yielding a lower limit $\langle B^2 \rangle_{min}^{1/2} =$ 1.1×10^6 G, if $\alpha = 1$.

If the earlier identification concerning the significance of the 12.2-day variation in the measurement of solar oblateness is assumed the value τ ought to be reduced to 12 days and $2\pi\Delta\nu\tau > 1$ yields $\langle B^2\rangle_{\rm mm}^{1/2} = 1.7 \times 10^6$ G, if $\alpha = 1$.

The observable effects of the magnetic field are, of course, weighted over the solar interior and this would be expressed in the size of the factor $\alpha_{n,l,m}$. In the absence of such model calculations one might put $\alpha_{n,l,m} = 1$ as a crude approximation and weight the square of the magnetic field in the solar interior $B^{2}(r)$ with a weighting function of the form of equation (1). Rough calculations show that the resultant is an observable mean square magnetic field which is smaller or larger than the volume averaged $B^{2}(r)$ depending on whether the field is extended or decreases rapidly (for example, as the matter density) with radius.

Speculations concerning large internal magnetic fields have been made before⁹⁻¹¹ but this is the first clear empirical evidence pertaining to the interior of the Sun which suggests their presence.

Such intense fields presumably have a fossil origin⁹. Although such fields are but a small fraction of the maximum fields allowed by the extended virial theorem¹², the much higher angular velocities of young solar type stars¹³ suggest that this may not have been the case in the early evolutionary stages of the Sun and presumably other main sequence stars. The magnetic flux, and therefore the internal field, is likely to be decreasing with time because of ohmic dissipation and also because of flux loss and angular momentum loss through the surface layers through the solar wind^{10,14,15}. In the early life of the Sun the magnetic energy may have been a non-negligible fraction of the total energy of the star.

Although the magnetic fields in the solar core considered above are large their contribution to the pressure in the core is likely to be too small to be significant in the context of the solar neutrino problem¹⁶.

The mixing that such magnetic fields are likely to provide 10 will, however, probably also solve the problems of the missing solar neutrinos 17,18 as well as the Earth's climatic problems caused by an ancient cool Sun19.

The asymmetric oblique magnetic rotator has a nutation frequency ω given by

$$\frac{\omega}{\Omega} \simeq \frac{E_{\rm M}}{|E_{\rm G}|} = \frac{1}{\Delta \bar{\alpha}} \frac{\delta(\overline{\Delta \nu_{nl}})}{\nu_{nl}} \leqslant \frac{1}{\Delta \bar{\alpha}} \frac{0.6 \text{ } \mu\text{Hz}}{3 \text{ mHz}} \leqslant \frac{1}{\Delta \bar{\alpha}} 2 \times 10^{-4}$$

where Ω is the angular velocity of the core taken to be $6\times$ 10⁻⁶ s⁻¹. Thus the period of one nutation should be longer than 166 $\Delta \alpha$ yr and as $\Delta \bar{\alpha}$ is likely to be <1 it is tempting to speculate, together with Dicke²⁰, that this corresponds to the 22-yr solar cycle. With this identification, the magnetic energy of the Sun would be increased above the value given by equation (4) and $\langle B^2 \rangle^{1/2}$ would be 9×10^6 G.

Other aspects of solar activity which seem to be explained by the above model will be discussed elsewhere.

If the above ideas concerning internal magnetic fields correspond to reality the Sun will fit quite naturally into the sequence of young Ap stars to white dwarfs and pulsars. Moreover the Sun will be able to sustain a very much richer spectrum of modes of vibration and it is conceivable that the 160-min oscillation^{21,22} is such a magnetohydrodynamic mode.

The magnetic effects should give rise to asymmetries which will be detectable with the higher resolution of longer strings of velocity measurements in the near future and may also produce periodic displacements with 12.2 or 6.1-day periods.

If the Sun has substantial magnetic fields in the core it is tempting to speculate that other stars also do and it is suggested that the β Cephei stars, for example, show large magnetic rather than rotational splittings and the magnetic fields may destabilize the stars sufficiently²³ to make them pulsate.

I thank Professor H. H. Voigt for the hospitality and the Akademie der Wissenschaften for support during my stay in Göttingen.

Received 1 September, accepted 7 December 1981

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Inferring solar UV variability from the atmospheric tide

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A new method of determining variations in the solar UV output is presented here. The method is based on the fact that the semi-diurnal (12 h) atmospheric tide is dominated by the component forced by ozone absorption of UV. This tide shows very little interannual variability; hence a variation in UV of 20%, as suggested by Heath and Thekaekara¹, would have an easily discernable effect on the tide. It can be shown that a change of 20% in UV would result in a 12% change in the semi-diurnal tide. The failure to find any solar cycle-related variation in the semi-diurnal tide suggests that the UV varies by <2% over the sunspot cycle.

The suggestion that the solar UV output varies by ~20% over the 11 yr sunspot cycle is controversial^{2,3}. Such changes in UV would alter ozone concentrations, temperature and wind in the stratosphere^{4,5}. This may affect the vertical propagation of planetary waves in winter^{6,7} and hence the troposphere. One indicator of ozone layer heating whose variation over the sunspot cycle is on record, but which has received little attention. is the semi-diurnal (12 h) atmospheric tide. Unlike oceanic tides, this is due to the heating effect of the Sun8. The dominant component is from ozone absorption of UV 8.

The model of atmospheric tides used here is that of Chapman and Lindzen⁸. The altitudinal and latitudinal dependences are separated. The latitudinal variation of the tides are Hough functions and the vertical structure equation, with appropriate boundary conditions, determines the magnitude and phase of each component of the tide at all levels. The effect of zonal winds and the interaction between different tidal components are ignored, which results in an underestimate of the amplitudes 5,10. The only forcings considered were the heatings due to ozone and water vapour absorbing solar radiation. The heating profiles were obtained using a model similar to that of Forbes and Garrett11.

Data were obtained from two sources of 'hourly averaged annual pressure', that is, the mean pressure at each hour of the day averaged over 1 yr. Results for Macau were from Resultados das Observações Meteorológicas, 1952-78, while the Indian values were from Indian Weather Reviews, Annual

Table 1 Details of the tropical stations

Station	Loc	ation	Years used	Total (yr)
Bombay	18.9° N	72.8° E	1948-54, 1956-62	14
Calcutta	22.5° N	88.3° E	1948–54, 1956–63	15
Macau	22.2° N	113.6° E	1952-1975	24
Madras	13.1° N	80.3° E	1951-54, 1956-61	10
Nagpur	28.6° N	79.1° E	1949-54, 1956, 1948-63	13
New Delhi	18.5° N	77.2° E	1949-53, 1956-63	13
Poona	4.6° S	73.9° E	1948-54, 1956-62	14
Seychelles		55.5°E	1951–54	4

Summaries, 1948-63. Table 1 lists the eight stations used, their locations and the years for which their data were available.

The amplitudes of the mean tides, averaged over the 107 station years, are 0.809 mbar (diurnal) and 1.180 mbar (semidiurnal). These are larger than the values the theory predicts, as a result of ozone and water vapour forcing alone, by factors of 5 and 1.6 respectively. This result is consistent with that of previous work in atmospheric tidal modelling^{8,10}. The causes of the disagreement between theory and observation are the approximation of linearity in the theory^{9,10}, and the neglect of local tropospheric heat sources^{12,13}. The latter is particularly evident in the diurnal tide, which is much larger over the continents than over the oceans.

Having obtained results for mean conditions, the heating profile is then altered by changing UV and ozone concentrations to examine the effect of UV variability. The ozone concentration is altered, due to changes in UV, by the amount predicted by stratospheric models^{4,5}. This has a negligible effect on the tides, as there is complete absorption of UV by ozone. The UV variation over the sunspot cycle is parameterized by a $\pm 10\%$ variation in the ozone heating in the Hartley Band, Huggins Band and Hertzberg continuum. The model predicts a change of $\pm 6.2\%$ in the semi-diurnal tide in the tropics, while the diurnal (24 h) tide would vary by $\pm 0.8\%$. It is assumed that the component of the tides unexplained by the theory has the same fractional change with UV variation as the theoretical component. As the semi-diurnal tide shows very little annual variation, a change of 12% would be readily visible.

Table 2 The mean tide, and the predicted and observed increase in the amplitude from sunspot minimum to sunspot maximum

	Diurnal (µbar)	Semi-diurnal (µbar)
Mean	809	1,180
Predicted	14	148
Observed	-41 ± 13	5±11

Results are from the stations listed in Table 1. The error estimates are one standard error.

The results for all the stations are considered together to reduce the standard errors. They are normalized at each station, before averaging over all the stations, so that changes solely in amplitude add together linearly and not vectorially.

The results are shown in Table 2 which highlights the discrepancies between the theoretical predictions and the observations. The diurnal tide shows a much larger variation (-41 \pm 13 µbar) over the sunspot cycle than predicted (14 µbar). The semi-diurnal tide, on the other hand, is predicted to increase by 48 μ bar but is observed to increase by only $5 \pm 11~\mu$ bar. This latter result agrees with previous work¹⁴, where no correlation was found between sunspot activity and the amplitude of the semi-diurnal tide at Batavia (for 1866-1945) and at Manila (1890-1938).

The variation in the diurnal tide is actually larger than the whole component forced by ozone heating, thus it is unlikely to be due to variations in UV. Hence, between 1948 and 1975 there was a diurnally varying tropospheric parameter which had a significant change over the sunspot cycle. However, where a significant change was expected, in the semi-diurnal tide, none was found. The observations of this tide provide an estimate of the maximum value for the sunspot cycle variation in UV. As shown above, a 20% change in the UV would cause a 12.3% change in the semi-diurnal tide, so assuming linearity, the observed tidal variation is due to a $0.5\% \pm 1.6\%$ variation in the UV. This result is more precise than any derived using conventional techniques³. Analysis of a larger surface pressure data set may lead to even greater precision.

This result shows that the atmospheric semi-diurnal tide can be used to examine possible solar UV variations. Indeed, this tide may also be usable for remote sensing of the stratosphere, in a way analogous to those for examining the internal structure of the Earth¹⁵ and Sun¹⁶. If this is the case, the large exant quantity of hourly surface pressure data might be used to determine possible stratospheric variations (unrelated to solar activity) over the past 100 yr.

I thank other members of the Atmospheric Physics Group at Imperial College for their support, particularly Dr J. S. A. Green, also the Meteorological Office Library for providing data. The work was financed by the NERC and the Appleton Laboratory.

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Diurnal variation of mesospheric ozone

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Photochemical models¹⁻³ predict a considerable variation in ozone concentration between day and night in the mesosphere. To investigate this, four Petrel rockets were flown from South Uist (57°22'N, 7°22'W) on 2 October 1979. Ozone was measured by observing the atmospheric attenuation of a narrow band of UV radiation, using interference filters to define a bandwidth of ~10 nm. The rockets were launched (1) at moonset, 02,00h, (2) at sunrise, 06,00h, (3) at 09,30h and (4) at 15.30h; round 1 observing moonlight and the other three observing sunlight. The first two used the occultation technique and contained two photometers with wavebands centred around 265 and 290 nm (Fig. 1), giving ozone information from 48 to 95 km. For rounds 3 and 4 the Sun was at a zenith angle of 70°, and a single waveband centred around 265 nm gave a height range of 44 to 64 km. Full details of the experiment will be published elsewhere. The first three rounds were successful, but the fourth did not give good data because the rocket nosecone failed to clear the field of view. The results show significant diurnal variation above 54 km, which exceeds a factor of 2 above 65 km and reaches a factor of 10 between night time and sunrise at 90 km.

To retrieve ozone density from the raw flight data, a profile was produced of attenuation as a function of height for each sensor. The signals near apogee showed no drift in detector sensitivity, but rounds 2 and 3 showed some dependence on rocket attitude (coning modulation). The observations near apogee were used to derive corrections for this, and to deduce the unattenuated signals for the rest of the flights. The attenuation profiles were completed by using a radar track of the first 64 s of flight, extrapolated using a ballistic trajectory, to give rocket height.

For zenith angle 70°, the transmission $\xi(h)$ at height h may be written

$$\xi(h) = \frac{\int d\lambda I_0(\lambda) S(\lambda) \exp{-\{\sigma_{ox}(\lambda) N_{ox}(h) + \sigma_{R}(\lambda) N_{aur}(h)\}}}{\int d\lambda I_0(\lambda) S(\lambda)}$$
(1)

where I_0 is the unattenuated source irradiance at wavelength λ , $S(\lambda)$ the sensor sensitivity, $\sigma_{cx}(\lambda)$ the ozone absorption coefficient and $N_{cx}(h)$ the path total of ozone molecules between rocket and source. σ_R and N_{air} refer to Rayleigh scattering by air molecules. This equation may be solved for each value of ξ to obtain $N_{ox}(h)$, and the number density $n_{ox}(h)$ (given by

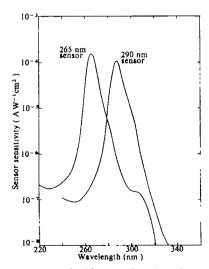


Fig. 1 Photometer sensitivities as a function of wavelength for round 2.

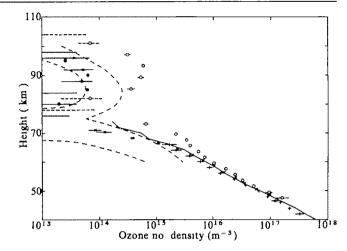


Fig. 2 Ozone number densities as a function of height. Error bars are random standard errors for the present measurements, and χ is the zenith angle of the centre of the Sun or Moon, viewed from the rocket. \bigcirc , 02.00h, $\chi = 94.5$ (Moon); \times , 06.00h, $\chi = 95.5$ (Sun); +, 09.30h, $\chi = 69.7$ (sun); —, daytime from ref. 14; \bigcirc , sunset from ref. 4; \bigcirc -, envelope of night-time values from ref. 16.

 $-\cos\theta \, dN_{cx}/dh$ for zenith angle θ) may then be obtained by differentiating equation (1) with respect to h. The raw attenuation values for round 3 were smoothed by fitting a quadratic curve over 4-km intervals (about 30 individual observations), which also gave $d\xi/dh$ with its statistical standard error.

For rounds 1 and 2, the attenuation profile was first expressed as a function of tangent ray height. Because of the finite angular size of the Sun and Moon the attenuation at the rocket was a smoothed version of that from a point source. $N_{\rm oz}(h)$ was retrieved at discrete levels assuming constant scale height between levels, by an iterative process similar to that described by Miller and Ryder⁴ and Miller⁵. Standard errors in $N_{\rm oz}(h)$ were obtained by calculating the covariance matrix.

To deduce number densities from the path totals, ozone was assumed constant in a series of concentric layers, thus the path totals N(h) could be written ${}_{1}\Sigma L_{1}(h)n_{1}$ where $L_{1}(h)$ is the length of the path through the *i*th layer and n_{1} the number density there. The inversion of this problem is very sensitive to noise in N(h) and the method described by Rodgers⁶ was used to stabilize the solution without introducing appreciable bias. This involved using an 'a priori constraint' composed of a hand-smoothed version of an unconstrained retrieval. Below 70 km, ozone number densities were retrieved for 1 km layers but to stabilize the solution above 70 km, layers 4-km thick had to be used. In each case standard errors were obtained by calculating the covariance matrix.

For each sensor, the solar spectrum used was that of Broadfoot⁷ below 300 nm and Simon⁸ for wavelengths above 300 nm. Ozone absorption coefficients were taken from Inn and Tanaka⁹, corrected for temperature after Vigroux¹⁰. Rayleigh scattering was calculated using Penndorf's¹¹ value for cross-section, and the atmospheric profile provided by the Stratospheric and Mesospheric Sounder (SAMS) on the Nimbus 7 satellite (J. J. Barnett, personal communication). Solar limb darkening was calculated from data given by Moe and Milone¹².

Round 3 produced only one ozone profile, on the ascent leg, but rounds 1 and 2 also gave profiles on the descent leg. However, because of the need to extrapolate the radar data beyond 64 s, there is considerable uncertainty in the rocket position on the descent, and data from the ascents only are presented, with a height uncertainty of ~ 250 m. (These profiles were in good agreement with those of the descents.) The results are presented as number densities in Fig. 2 and mixing ratios in Fig. 3, atmospheric density being calculated from SAMS temperatures, extended above 75 km with the CIRA $(1972)^{13}$ standard atmosphere for 60° N. Values shown are from the 290 nm sensors below 64 km and from the 265 nm sensors

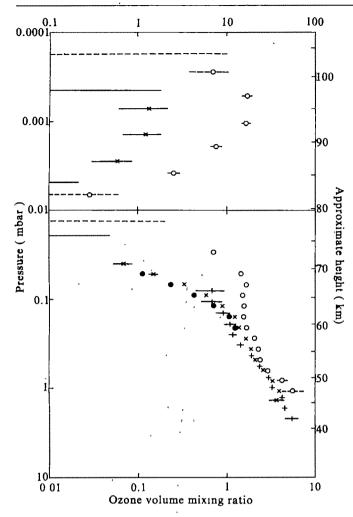


Fig. 3 Ozone mixing ratios as a function of pressure. Error bars are random standard errors. Note the scale change on the abscissa above 0.01 mbar. O, 02.00h; ×, 06.00h, assuming spherical symmetry in retrieval; •, 06.00h, allowing for ozone variation along the path; +, 09.30h.

above this height. For rounds 1 and 2 the height resolution is ~ 5 km, and for round 3, ~ 4 km.

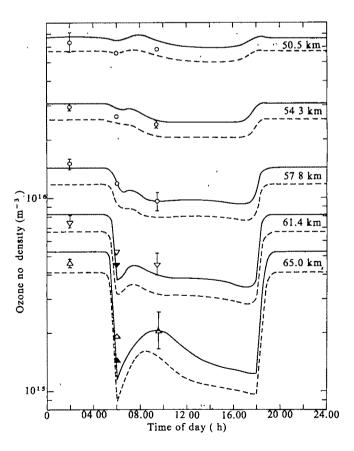
Errors in the ozone absorption coefficient (especially the temperature correction) and the spectral calibration contribute most of the systematic uncertainties in the results; the former amount to $\sim 4\%$ and the latter to 2% increasing to 5% below 48 km. Below 70 km for the dawn round, a test was made of the sensitivity of the retrieval to the hypothesis of horizontal uniformity, as ozone is predicted to vary rapidly near the terminator above 60 km. The photochemical model described below was used to predict the ratio of the concentration in each layer to its value at 90° zenith angle, and these ratios were incorporated in the retrieval. The differences from the original profile are shown in Fig. 3—they increase from $\sim 5\%$ at 58 km to a maximum of 28% at 67 km.

The results show a significant day-night difference in ozone concentration above 53 km, which reaches a factor of 10 above 70 km. The retrieval technique was unable to give more than an upper limit on the ozone amounts in the 'dip' of the profiles around 78 km, but the large differences persist in the upper ozone layer around 90 km. The dawn and morning profiles agree with Krueger and Minzner's¹⁴ (45° N, daytime) standard midlatitude ozone model between 46 and 66 km, and the dawn values above 80 km agree well with those of Miller and Ryder⁴ (58° N, September, sunset) and Llewellyn and Witt¹⁵ (68° N, March, sunset, not shown on Fig. 2). The night-time measurements are compared above 60 km with the envelope of equatorial profiles obtained using stellar occultation by Hays

and Roble¹⁶. Unlike the present values, these show a divergence from the Krueger and Minzner¹⁴ model below 65 km, which is not supported by other workers^{17,18}. Above 65 km, our values are the greater at all altitudes, with a peak concentration 9 km higher, possibly due to the difference in latitude.

Previous measurements of the day-night difference of ozone below 65 km have been reported by Hilsenrath¹⁹ (38° N, March) and Anderson *et al.*²⁰ (50° S, December), both of whom show a factor of 2 at 65 km, in agreement with the present work. The change in column total above 50 km of 10¹⁶ molecules cm⁻² agrees with the measurement of Penfield²¹, even though the profiles he showed were very different from ours. Above 70 km, our night-time column total of $1.24 \pm 0.07 \times 10^{15}$ cm⁻ agrees well with that of Wilson and Schwartz²², who report $1.1\pm0.4\times10^{15}$ cm⁻². These authors, like Penfield, used groundbased microwave spectroscopy, which has the disadvantage of poor height resolution (~20 km below 70 km), precluding a direct comparison with our measurements at specific heights. However, they show no sign of the minimum in ozone concentration around dawn and small maximum in mid-morning above 65 km as predicted by theory (see below). In contrast, the present measurements show the dawn profile crossing that at 09.30 near 65 km, indicative of just such a feature.

Above 80 km, there have been no coincident measurements of day and night ozone profiles to compare with this work. Model predictions of day-night differences in this region vary considerably, and depend on the value adopted for the vertical diffusion coefficient¹. Hunt²³ predicts a night-time enhancement factor of 2 and Shimazaki and Laird² predict 100. Theoretical predictions below 70 km are less variable but a quantitative comparison between theory and experiment requires a model run at the same latitude with the same profiles of relevant atmospheric variables. Comparison will therefore be made with



Flg. 4 Comparison between observations and theory Solid lines are theoretical calculations with 2 p.p.m. water vapour and the broken lines correspond to 4 p.p.m. water vapour. Open symbols at dawn refer to retrievals assuming symmetry and closed symbols refer to retrievals with variable ozone (see text and Fig. 3).

a one-dimensional model adapted from the Oxford University two-dimensional model²⁴. This integrates the photochemical continuity equations diurnally (with no transport processes) for the odd oxygen, hydrogen, nitrogen and chlorine active species and their associated reservoirs, using rate constants as given in JPL 81.3 (ref. 25). A basic timestep of 5 min (less around dusk and dawn) is used for levels half a scale height (~4 km) apart in the vertical. For this work, the solar spectrum and absorption coefficients were based on the recommendations of Nicolet 26,27 with the parameterization of Frederick and Hudson²⁸ being used for water vapour photodissociation. Temperatures were taken from SAMS data, the latitude fixed at 57° N and the time of year at equinox. A full description of the modelling work will be published elsewhere.

Two sets of model calculations, corresponding to water vapour mixing ratios of 2 and 4 p.p.m., are compared with the observations in Fig. 4. The basic day-night difference in ozone above 55 km is caused by the recombination of atomic oxygen to form ozone at dusk $(O+O_2+M\rightarrow O_3+M)$ and its formation by ozone photodissociation at dawn. The small peak in midmorning above 60 km results from the depletion of water dissociation products (H, OH, HO₂) during the night and their slow release from water during the morning; these products are the main destruction agents for odd oxygen in the mesosphere.

The agreement between theory and observation is consistent with a water vapour mixing ratio profile falling from ~ 3 p.p.m. at 50 km to \sim 2 p.p.m. at 65 km. The magnitude of the diurnal variation (which is relatively insensitive to the water vapour) is overestimated at the upper levels by $\sim 15\%$, possibly due to uncertainties in the rate constants used—the quoted uncertainty²⁵ for the rate of $O+O_2+M\rightarrow O_3+M$, for instance, is enough to account for this discrepancy. It may be concluded, then, that the observations are in agreement with current photochemical theory (given the uncertainty in the latter) provided that mesospheric water vapour abundances of 2-3 p.p.m. are accepted. Recent measurements of this quantity have varied from 1.5 p.p.m. (ref. 29) to 15 p.p.m. (ref. 30) at 60 km, and this work supports a mixing ratio near to the lower end of this

I thank J. H. Seymour for work on the design and preparation of the experiments, also S. G. Palmer and F. H. Price for contributions to the data analysis, and D. E. Miller for support and advice.

Received 25 September 1981, accepted 19 January 1982

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'Paradoxical' mechanics under fluid flow

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Interest in the fluid loading of engineering structures has been stimulated by acute technological problems arising in the exloitation of off-shore oil fields. Here slender riser pipes, and complete tethered-buoyant platforms must be designed against potentially dangerous instabilities. We show here how conventional structural theorems can be not only violated, but actually reversed under fluid loading due to its essentially non-conservative character. Under increasing flow, for example, the stiffness of an elastic structure can increase, pass through infinity, and become negative. Surprisingly, the negative stiffness domain is stable, but can be destabilized by the addition of a constraint. Experiments on a hanging articulated pipe conveying fluid nicely confirm the theory.

Suppose we are given a black box with a tray hanging beneath it. We add a small mass m to the tray, and it rises a small ce h: we add a second mass m and it rises a further distance h. To our surprise, we find that we can plot a complete stable load-deflection curve with negative stiffness using dead weight loading. What is in the box?

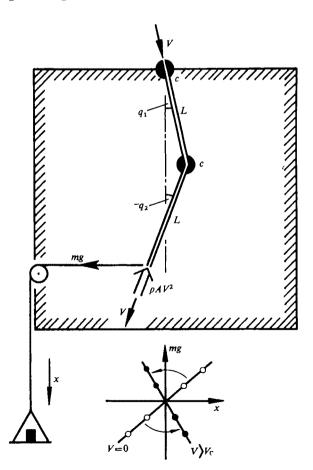


Fig. 1 Articulated pipe system in a box, exhibiting stable negative stiffness under dead loading. The specific details of the system relate to the theoretical study and are not essential. The lengths and stiffnesses need not be the same, and the phenomenon can indeed be exhibited by a continuously flexible hanging hose pipe.

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Now a local negative stiffness is quite common in structural mechanics, being, for example, generated by an elastic structure that has lost its initial stability at a fold or limit point: but it is then always associated with unstable equilibrium state under dead load. Indeed, an inverted rigid pendulum in our black box would give an equilibrium path of negative stiffness, but the path would be unstable under dead load: it could only be stabilized and therefore studied experimentally by using a suitable rigid-screw loading device.

We can be quite sure, by a fundamental theorem of structural mechanics¹, that our mysterious black box certainly does not contain a conservative mechanical system.

The answer is that it contains an articulated pipe carrying an internal fluid flow, as studied by Benjamin². Two straight and rigid pipes of length L are pinned elastically to one another and to a fixed support by rotational springs of stiffness c, forming a sort of cantilevered hose pipe, the fluid discharging with velocity V into the atmosphere at the free tip.

The statics of the non-conservative system are no mystery. Ignoring gravity, which is inessential in our present theoretical demonstration, the centrifugal force at the knee is statically equivalent to a follower force of magnitude ρAV^2 at the discharging tip as shown in Fig. 1. Here ρ is the density of the fluid and A is the internal cross-sectional area of the pipes. So for small deflections from the vertical, the linearized equilibrium equations are easily written down in terms of the small angles q_1 and q_2 . Moments about the knee for the lower link give

$$mgL + c(q_2 - q_1) = 0$$

and moments about the support for the whole system give

$$2mgL + cq_1 + FL(q_2 - q_1) = 0$$

while the corresponding deflection of mg is

$$x = -L(q_1 + q_2)$$

Here g is the acceleration due to gravity, and $F = \rho A V^2$. Solving these we find

$$x = -mgL^2(2\rho A V^2 L/c - 5)/c$$

so the flexibility x/m decreases linearly with V^2 passing from positive to negative as V passes through its critical value $V_{\rm C}$

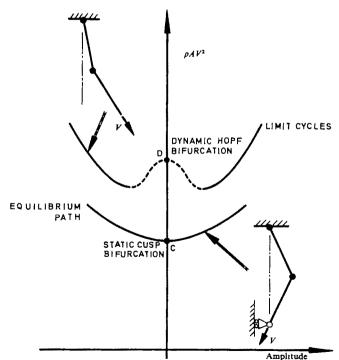


Fig. 2 Buckling (divergence) of a constrained system before the flutter of the corresponding unconstrained system. The dynamic flutter bifurcation is locally sub-critical, but the emerging unstable limit cycles are subsequently stabilized at large deflections.

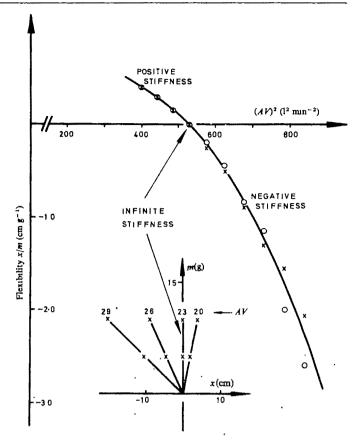


Fig. 3 Experimental results showing a region of stable negative stiffness under dead loading. Infinite stiffness occurs at a critical value of the flow velocity V.

given by setting the expression in brackets to zero. The stiffness m/x therefore increases with V, passes through infinity at $V = V_{\rm C}$ and becomes negative for higher values of V. In all of this, it is to be understood that V is held constant during the stiffness measurements.

So provided the flow velocity is greater than V_c , we have our negative stiffness. The statics is thus easily understood, but why is the system stable at these high velocities? The answer lies in a complete dynamical stability analysis following Benjamin². The displacement-dependent 'follower' force F at the tip is a circulatory force³, being non-conservative and not derivable from a potential energy function: it is represented by a non-symmetric matrix in the lagrangian equations of motion. In addition, the fluid generates velocity-dependent Coriolis forces.

The dynamical study shows that the system is indeed stable for flow velocities up to and above $V_{\rm C}$, stability finally being lost at a dynamic instability at a higher velocity $V_{\rm D}$. This dynamic bifurcation to flutter is of the Hopf type, and on the basis of Lunn's new experiments⁴, the emerging limit cycles are seen to be unstable in the vicinity of the branch, with the form of Fig. 2.

The paradoxical static behaviour arises before the onset of this dynamic instability for $V_C < V < V_D$. It is related to the fact, noted by Benjamin², that if the free end were pinned (but free to move vertically) the resulting single degree of freedom system would buckle (diverge) statically at a flow velocity of $V = V_C$. This single degree of freedom pinned system exhibits a stable-symmetric point of bifurcation (stable cusp) at V_C as shown in Fig. 2, analogous to the behaviour of a continuous pin-ended pipe⁵.

Note here a second paradox. When $V_C < V < V_D$ the addition of a constraint $(q_1 + q_2 = 0)$ destabilizes the system. This cannot occur in a conservative mechanical system. It was observed experimentally by Benjamin² and repeated by Lunn⁴: if the finger is pressed very lightly against the side of the discharging

tip, the pipes buckle like the pinned system pressing strongly and unexpectedly against the finger.

This brings us to the final paradox. The finger is acting like a semi-rigid screw loading device, under which the system is unstable. In the same flow conditions the pipes are stable under dead loading. This is the exact converse of the behaviour of a buckled elastic structure which is stabilized by semi-rigid loading1

Experiments confirming these theoretical deductions are shown in Fig. 3. These used Lunn's experimental pipes, and show the flexibility decreasing with the square of the flow velocity, although not quite in the linear manner of our simple theory which neglected gravitational effects. Perfectly stable equilibrium paths of negative stiffness were obtained in deadloading conditions. The infinite stiffness at $AV = 23 \,\mathrm{l\,min^{-1}}$ is achieved by the structure buckling progressively in its single degree of freedom mode for which $q_1 + q_2 = 0$, thereby giving the reaction ρAV^2 greater and greater leverage about the top support. It is, of course, eventually destroyed by large deflection nonlinear effects.

This new concept of stable negative stiffness explains the strange behaviour discussed qualitatively by Benjamin, and has important implications for the stability and strength analysis of the flexible marine structures now being proposed.

Received 26 November 1981; accepted 20 January 1982

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Structural effects in electrocatalysis

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Studies of chemical reactions on well-defined surfaces provide fundamental data on surface reactivity and guidelines for the understanding and design of catalytic materials but few such studies have been done in electrocatalysis. We report here a study of the oxidation kinetics of HCOOH, CH₃OH and CH₂O on single crystal platinum electrodes with (100), (110) and (111) orientations. Pronounced dependence of the kinetics of these reactions on the crystallographic orientation of the surface has been found. The potential regions where reactions take place, the peaks of voltammetry curves, and the magnitude of currents at the peaks are different for each plane. These differences are explained on the basis of adsorption of a strongly bound intermediate, which shows a pronounced dependence on the symmetry of the single crystal planes. This intermediate completely blocks the Pt (100) surface; the smallest adsorption is at the (111) plane. On activation, the (100) surface shows the highest activity. These results suggest that electrocatalytic reactions exhibit structural sensitivity. They also provide guidelines for designing catalysts for the oxidation of small organic molecules to be used in electrochemical energy conversion.

Kinetics of typical electrocatalytic reactions have been studied on poorly characterized high-area supported electrodes and polycrystalline electrodes. Only a few electrosorption reactions have been studied on single crystal surfaces which include hydrogen adsorption¹⁻⁴ and ad-atom formation (underpotential

deposition of metals)⁵. Previous work on the oxidation of HCOOH, CH₂O and CH₃OH has indicated that these reactions 'site-demanding' reactions, requiring specific lattice geometries. Bagotzkii et al.6 showed that in the oxidation of CH₃OH three sites are engaged in adsorption of the intermediate COH. Such reactions will be expected to be sensitive to the structure on (111), (110) and (100) surfaces. The role of specific multiple site requirements was introduced by Balandin⁷ as early as 1929.

The method of preparation and characterization of single crystal surfaces have been described elsewhere8,9. The electrodes were prepared from a Pt single crystal obtained from Materials Research Corporation. The crystals were oriented by Laue back scattering to better than 0.5°. The samples were cut and polished using standard metallurgical techniques and were then slightly etched in boiling aqua regia to remove the work hardened surface. The samples were then annealed at 900 °C for 3-4 h in pure oxygen, reduced at 900 °C for 30 min in hydrogen and cooled over a period of 12 hours in this same atmosphere. They were mounted in Teflon tubing. Immediately before the electrochemical experiment, the electrodes were exposed to 3M HClO₄ to remove possible contaminants such as CaO; they were then rinsed several times with triply distilled H₂O. The electrolyte was 1M HClO₄ prepared from Merck HClO₄ and H₂O prepared by a pyrocatalytic distillation.

Figure 1a shows voltammetry curves for the oxidation of formic acid for three single crystal orientations of Pt. The curves differ substantially in shape and in the magnitude of the currents associated with various peaks. The sweeps in the anodic scan, that is the curves obtained by increasing the potential, show that the highest activity is obtained with the (111) and lowest with the (100) orientation. At the Pt (111) surface the reaction commences at the most negative potential. At the (100) surface the one peak seen sweeping in an anodic direction is usually ascribed to the oxidation of a strongly bound intermediate which blocks the electrode surface for further oxidation of HCOOH. Its oxidation coincides with the onset of oxide formation (100) plane. After activating the surface is this way, the curve obtained by decreasing the applied voltage shows much higher activity. No such effect is seen with the Pt (111) surface: the sweeps almost retrace themselves. This suggests that very little, if any, of the strongly bound intermediate is formed on the (111) plane. The Pt (110) surface shows an intermediate behaviour. Note that the peak in cathodic direction appears at potentials much more positive than the corresponding peak for the (100) plane. Important information is obtained when these curves are compared with that of a polycrystalline electrode. The peak contributions could be correlated with the contributions of the three low Miller index planes. Figure 1b shows a typical voltammetry curve for the oxidation of HCOOH on a polycrystalline platinum electrode. A comparison of the peak potentials on this curve with those obtained for three single crystal orientations (Fig. 1) shows that the first peak in anodic scan (\sim 0.48 V) is characteristic of the (111) plane, and to a lesser degree, of the (110) surface. The second peak in the anodic scan (~0.9 V) is characteristic of the (100) and (110) surfaces. In the cathodic direction, the first peak at 0.7 V is due to the reaction at the (110) surface, while the shoulder at ~0.4 V is characteristic of the (100) plane.

Figure 1c displays voltammograms obtained with methanol. The oxidation commences at 0.5 V at the (111) plane. At the (110) and (100) surfaces the reaction starts at 0.68 and 0.75 V respectively. The largest current is associated with the peak at 0.8 V on the (100) plane. However, from a practical point of view this oxidation takes place at too high a potential because a cell with such electrode would have too low voltage. In that respect, the (111) plane could be considered as the most active catalyst. Note that CH₃OH affects hydrogen adsorption on Pt to a lesser extent than do CH₂O or HCOOH. These data are at variance with the work of Bagotzkii et al.12, who did not find any significant difference between the activity of these single crystal electrodes.

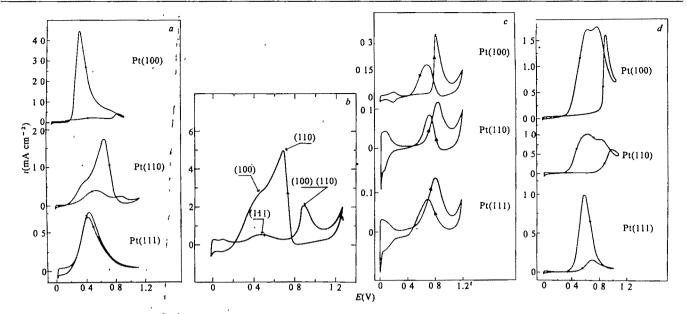


Fig. 1 a, Oxidation of HCOOH (0.26 M) on single crystal Pt electrodes with (100), (110) and (111) orientations in 1 M HClO₄. b, Oxidation of HCOOH on polycrystalline Pt electrode. Assignments of peaks are given in the graph. c, Oxidation of CH₃OH (0.26 M) on single crystal pT electrodes with (100), (110) and (111) orientations. d, Oxidation of CH₂O on single crystal Pt electrodes with (100), (110) and (111) orientations. Sweep rate 50 mVs⁻¹, temperature 22 °C.

The reaction for formaldehyde begins first at the Pt (111) surface, (Fig. 1d) at E = 0.4 V and reaches a peak at E = 0.75 V. At the Pt (100) surface the oxidation commences significantly only at 0.75 V, with the peak at 0.88 V. The current associated with this peak is, however, higher than the current for the (111) and (110) surfaces. After activation of surfaces at the most positive potentials, the sweeps in the cathodic direction show the highest activity on the (100) plane. This shows that strongly bound intermediates in the oxidation of CH2O on Pt are predominantly formed on the (100) and (110) planes.

It is usually assumed that in the oxidation of HCOOH, CH₃OH and CH₂O, which should lead to CO₂, the same strongly bound COH intermediate is formed which blocks the surface, thus decreasing its catalytic activity. Differing activities of the (111), (110) and (100) Pt single crystal electrodes are ascribed to different degrees of adsorption of COH species. Adsorption is lowest on the (111) surface and highest on (100) plane, with (111) orientation generally exhibiting low reactivity. Low hydrogen adsorption on the Pt (111) surface, found by most authors¹⁻³, also has a role in determining the activity of this surface for the oxidation of HCOOH, because adsorbed H participates in the formation of COH. The adsorption and reduction of CO₂ on Pt, which gives the same species, COH, also gives the smallest coverage on the (111) surface 13. This corroborates the above conclusion. Crystallographic anisotropies of the above reactions in many ways mimic those found in the gas phase for CO adsorption¹⁴ on the same crystal planes. CO is the species closest in character to COH, which provides the basis for the comparison of these data.

There are no corresponding studies of adsorption and dehydrogenation of HCOOH, CH₃OH and CH₂O on Pt single crystals. As in the case of CO, the anisotropies which we have found cannot be predicted on the basis of theories of adsorption15 and electrode reactions16 presently available.

On the basis of these data we conclude that the electrochemical oxidation of HCOOH, CH₃OH and CH₂O on Pt exhibits significant crystallographic anisotropies. As these are typical electrocatalytic reactions, which involve a strong adsorption before the charge transfer, these data strongly suggest that most such reactions should show a pronounced structural sensitivity.

Note added in proof: Since submission of this paper, Clavilier et al. 17 have reported similar results.

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Controlled nucleation and quasi-ordered growth of ice crystals from low temperature electrolyte solutions

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The process by which ice forms during the cooling of water and aqueous solutions has been studied intensively mostly in conditions where the nucleation occurs heterogeneously-on some particulate impurity surface. When the more fundamental homogeneous process has been deliberately investigated 1-3 it has nearly always been in conditions in which the crystallization

process, once initiated, occurs with great rapidity^{1,2}. Seeking an additional 'degree of freedom' in the investigation of this important phenomenon, we have initiated the crystallization in conditions where the growth is extremely slow and the nucleation events can be controlled. Our observations are described below.

Slow nucleation conditions are obtained by choosing a concentration of water in an electrolyte solution such that the homogeneous nucleation temperature, which may be obtained in separate experiments using emulsions^{2,3}, lies very close to the glass transition temperature. In these conditions the growth of fluctuations to become, first, nuclei and then observable crystals, occurs at a rate which may be varied at will by changing the temperature of observation within a narrow range of a few degrees on either side of the glass transition temperature⁴.

The study of nucleation of ice in such conditions has gained in interest since the observations by A.F.W. and colleagues⁵ of the manner in which crystallization patterns of inorganic high temperature glasses can be influenced by the preceding thermal history. It was demonstrated that, for the appropriate heat treatment, crystallites could be observed growing with correlated spatial distributions with crystallization centres as small as 20 Å in radius and mean separations in the range 200-700 Å depending on thermal history. Since the origin of this phenomenon was not clear at the time (1979), it was clearly of interest to determine whether similar ordered crystal growth could be observed in a different type of system. We therefore undertook a careful study of the development and distribution of crystallites of ice formed in an initially vitreous lithium chloride + D₂O solution, using the small angle neutron scattering (SANS) technique. The conditions for glass formation, and observation of diffusion-controlled nucleation, have been described elsewhere4.

Solutions of 10.5 mol% lithium chloride in D_2O (6.17 m LiCl) were prepared in controlled conditions to avoid the ingress of any light water, H_2O . Samples of the solutions were introduced into a 3-mm space between sapphire windows in a cell whose temperature could be manipulated by a controlled temperature block in which the cell was mounted. The block with cell was then suspended in a liquid helium cooled cryostat which provided the low temperature ambient for the temperature control system. The temperature of the solution was taken to be that of the sapphire window at the edge of the cell, as preliminary experiments had shown that a gradient of <0.1 °C existed between the centre and edge of the sample. The temperature of the cell was monitored during the experiment by a fine chromel/constantin thermocouple in good thermal contact with the window.

The sample was initially vitrified by quenching into liquid nitrogen placed in the bottom of the cryostat and monitoring the temperature to ensure that it did not fall below 130K. Under these circumstances no cracks developed in the sample: the sample remained completely transparent to visible light and caused very little scattering of neutrons. (In some cases where the temperature drop was inadequately controlled, and cracks due to mechanical stress developed, the effects (observed as an excess scattering), could be seen to disappear during the first 2 h of annealing at 139K.)

On the basis of previous observations⁵ of the effect of annealing below $T_{\rm g}$ on the kinetics and spatial characteristics of nucleation, and with the assistance of preliminary calorimetric and light-scattering studies, it proved possible to determine thermal histories which would produce ordered distributions of microscopic ice crystals in our system. The conditions are critical, variations in choice of annealing temperature >1 °C not being allowed. Spectra showing the peak in Q described below were finally obtained using a nucleation temperature of 139 K (compare with $T_{\rm g} = 142$ K) for a period of 48 h, followed by observation at 140–142 K during which the nuclei which formed at the lower temperature grew to observable dimensions.

Following the annealing period, the sample still at 139 K was placed in the cold neutron beam of the D11 (low angle scatter-

ing) instrument of the ILL HFR (Grenoble). The wavelength of the study ($\lambda = 9$ Å) and the position of the multidetector (10 m behind the sample) were chosen so as to detect the correlations in the spatial distributions of the nuclei centres. With these conditions we obtained a range of scattered neutron wave vectors $2 \times 10^{-3} < Q < 3 \times 10^{-2}$ Å⁻¹. The counting time, 20 min, was a compromise based on the need to acquire reasonable statistics, while still following the time development of the system at a rate compatible with total allotted beam time.

At 139 K the sample gave only a very weak, featureless and time-independent scattered signal. This remained true while the temperature was raised slowly to 140.4 K. At this temperature, a statistically significant increase in scattering was observed, and the temperature increase was halted to permit isothermal observation of the sample time evolution (see Fig. 1). The scattered intensity which developed after 4 h is shown in Fig. 2a, and is notable for the intensity maximum at $Q_m = 0.9 \times 10^{-2} \, \text{Å}^{-1}$. This feature indicates some spatial organization of scattering centres, with a mean distance of separation of 700 Å.

After 6 h, the evolution effectively ceased, and to accelerate the development the temperature was raised (see Fig. 1). An increase in intensity and loss of peak definition was observed as the temperature rose to 143 K but thereafter an unexpected decrease in intensity occurred and continued until the signal virtually disappeared at 149 K (see Fig. 2b). The signal then reappeared, with a suggestion of a maximum in Q at the same value as before, and grew rapidly. This second increase was followed isothermally at 151 K (see Fig. 1) for 2 h. According to separate observations the sample, up to this point, remains optically transparent. Finally, observations were made during continuous temperature increase to T > 153 K, during which the sample becomes optically opaque, implying the presence of many large particles produced by ripening in the more diffusive conditions. Visible light scattering in the presence of a maximum in Q was also observable in microcrystallized cordierite glasses in the case where $\kappa_{\text{max}} < 1.5 \times 10^{-2} \text{ Å}^{-1}$ (ref. 7).

The observation of a maximum in the scattered intensity spectrum following annealing treatments similar to those used previously⁵ for silicate systems suggests that the ordering of crystallite centres has a common origin. The origin has been discussed elsewhere⁶ in terms of stabilization of crystallization centres initiated in highly metastable conditions in which the nucleation probability is high (because the critical nucleus radius is very small) and the diffusion length is small. The latter condition allows particles to grow independently of one another despite small separations if they lie beyond the region of steep concentration gradient established around each growing particle. New nucleation in the region of influence of each established

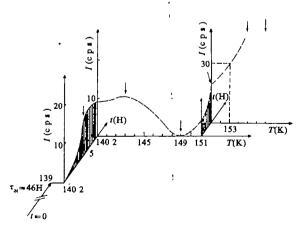


Fig. 1 Time-temperature-scattered neutron intensity schematic for this experiment. Note particularly the long annealing time at 139 K, the gradual development of scattered intensity with time at 140.2 K and the relatively sudden loss of intensity in the range 143-149 K. Short down-pointing arrows indicate conditions for the spectra shown in Fig. 2.

particle quickly becomes improbable in comparison with further growth of the established particle. Thus a preferred distance of separation, which is smaller the lower the temperature of nucleation, becomes established, and resists dissipation during the subsequent temperature increase to the value chosen for observation.

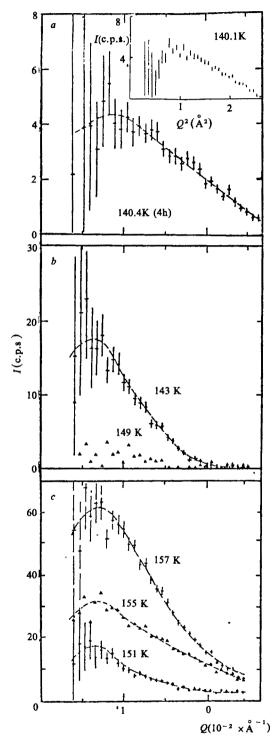


Fig. 2 Scattered neutron intensity in c.p.s. as a function of Q^2 at different stages of the experiment. a, After 4 h of development of 140.2 K. b, During temperature change in the range 143-149 K showing disappearance of signal. c, Development of signal with temperature increase between 151 K and 157 K. Note the maximum in I at $Q^2 \approx 0.9 \times 10^{-2} \text{ Å}^2$ in 140.4 K spectrum which persists throughout the experiment, with some movement to lower Q^2 values at higher temperatures. The same feature was seen better resolved (see insert to a) in an earlier exploratory experiment, for which the time-temperature history in the annealing range was less simple.

Considering the mean separation of crystallites of 700 Å, we observe that the nucleation density at 139 K must be at least 2.500 centres per um³—so high that the homogeneous nature of the nucleation phenomenon under study cannot be doubted. A Guinier plot of the high Q side of the peak yields an average crystallite size for the small particles, after initial development at 140.4 K, of ~100 Å. We can confirm approximately the size of particles which must be present by dividing the total ice volume which would form at complete desaturation (determined from the metastable extension of the ice saturation line in the phase diagram)4 by the number of scattering centres in the sample volume containing the precipitated ice. This calculation yields a maximum particle radius of 220 Å. Because our observation is made early during the desaturation process, an average radius of 100 Å seems reasonable.

The loss of scattering intensity at intermediate temperatures at first suggested that the initial scattering might have been due to a liquid-liquid phase separation which, as in the case of lithium disilicate⁷, produces conditions favourable for crystal nucleation. The new scattering centres which form in these conditions at a temperature above 149 K should produce a SANS spectrum unrelated to the initial pattern produced by the phase separation. That the renewed scattering pattern in the present case apparently retained the same maximum in Q as the initial scattering pattern suggests a more mundane explanation.

Neutron diffraction⁷ and calorimetric studies⁸ have now shown that the SANS phenomena observed result from a continuous development of microcrystals for T>140 K. This suggests that the peculiar variation in the scattered intensity for 143 K < T < 149 K (Fig. 1) probably originates in the relative variations in density and molar scattering power of the precipitated ice and the residual solution, which we can only assess approximately.

Combining data for the various isotopes, we find the scattering cross-section per mole of solution decreases rapidly with increasing concentration of LiCl. If, due to its large molar volume, the scattering power of ice initially formed from the supersaturated solution is weaker than that of the initial solution then, as the ice precipitates and residual solution concentrates, the contrast must pass through zero, giving a scattered intensity minimum. The low temperature density data for LiCl+D₂O solutions and D₂O ice necessary to confirm this hypothesis are not available, but extrapolations of LiCl+H₂O solution data, available down to -60 °C (ref. 9), show the postulated crossover is entirely possible. The explanation is also consistent with the relatively weak scattering observed at all temperatures.

Finally, the type of measurement described here provides a valuable basis for studies of other physical properties which, while sometimes more sensitive to the formation of the crystallizing phase8, contain no direct information on its size or distribution. These experiments and initial studies carried out at the eutectic composition, have produced no evidence in favour of a liquid-liquid phase separation occurring near T_{s} , either at the eutectic composition 10 or below 11

C.A.A. thanks the NSF for support of this work during a sabbatical leave from Purdue University at the Institut Laue-Langevin, France, under grant no. DMR77-04318A1. We thank D. Brochier and S. Pujol for help in the design and fabrication of the sample temperature control block, and J. P. Lelieur for preliminary calorimetric measurements.

Received 16 November, accepted 29 December 1981

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Setting of gabbroic dykelets in an ophiolitic complex by hydraulic fracturing

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Gabbroic dykelets cutting peridotitic tectonites are generally interpreted as the product of *in situ* partial melting, or as the path of upward moving magma. However, we report here that their structure strongly suggests emplacement by hydraulic fracturing, and their bulk and mineral chemistry indicate that they were highly differentiated liquids issued from the overlying magma chamber. They cannot be the feeding channels of the magma chamber, which contains the primitive liquids produced by partial melting of the upper mantle.

Within ophiolitic complexes, several types of dykelet (usually 1-5 cm wide, exceptionally up to 50 cm) can be observed in both the tectonite and the cumulate sequences. They are called dykelets to distinguish them from the isolated diabase dykes¹, which are ~2 m wide and cut throughout the ophiolitic sequence. Because the small dykelets have previously been interpreted as the feeding channels of the cumulate magma chamber¹²², in looking for the path and mechanism of feeding for the chamber we concentrated our investigations on the Antalya ophiolite complex (Fig. 1), which is particularly rich in internal structures related to ridge activity³.⁴.

This ophiolite consists of two well-exposed complexes (Fig. 1), the tectonic harzburgite massif of Adrasan in the south, and the massif of Cirali/Tekirova ~15 km farther north, consisting of harzburgitic tectonites and magmatic ultrabasic and basic cumulates. The coarse-grained texture ('S1') of the tectonites is due to high temperature, high-pressure, low-creep stress plastic flow below an oceanic ridge. However, in Adrasan and at the south end of the Cirali/Tekirova massif, shear zones with a porphyroclastic texture ('S2') are evidence of a low temperature, low lithostatic pressure, high creep stress deformation, obviously linked to intra-oceanic thrusting before obduction⁵⁻⁸.

A group of leucocratic gabbro dykelets can be observed among all dykelets occurring either in the tectonites (commonly deformed dunite and pyroxenite veinlets) or in the cumulates (nondeformed pyroxenite and gabbro dykelets) (I.R., in preparation). They never exceed 1% of the total rock, even in areas where they appear to be abundant, and may total $\sim 0.05\%$ of the massif. Their abundance in the tectonites decreases rapidly downwards in the upper 500 m.

These gabbroic dykelets neatly cross-cut all structures due to and affected by the plastic flow below the ridge, as well as the magmatic structures in the cumulates. Their mineralogy and chemistry are similar in both parts of the ophiolite, and quite different from those of the other dykelets. In fact, their bulk rock as well as their mineral chemistry (bulk rock chemistry analyses are representative of the total dykelet, as the sharp contact between dykelet and host rock permitted easy separation of the two, Figs 2, 3) correspond to those of evolved residual magmatic liquids, similar to the liquids which crystallized in residual pockets (Fig. 4e) in the upper part of the cumulate sequence. As Fig. 2 shows, there is a marked decrease in the MgO content and an increase in the Al₂O₃ content for the same SiO₂ and CaO contents, compared with those of the host plagioclase-wherlite or host gabbros of the cumulate sequence. The most differentiated dykelets are those found within the harzburgitic tectonites. This same trend occurs in the chemistry of clinopyroxenes and orthopyroxenes (Fig. 3), which show an enrichment in Fe content. Again, this enrichment is most important for the dykelets in the tectonites.

Thus, the leucocratic gabbro dykelets could not be the feeding channels of the overlying cumulate magma chamber. Their chemistry does not correspond to that of liquids produced by upper mantle partial fusion, nor to a possibly picritic parental magma, from which a thick layer of ultramafic cumulates could have crystallized, but it does correspond to the composition of residual liquids developed in the overlying gabbroic cumulates. Actually, the composition of MORB primitive liquids 9-12 corresponds to a mean value for the cumulates of Antalya (except for CaO values, which are quite dispersed anyway). However, the composition of the gabbro dykelets is considerably more evolved. Neither do they resemble that of the Cyprus upper pillow lavas¹³, believed to represent off-axis magmas¹⁴. When the Omanian gabbro dykelets, partly considered to be off-axis magma⁸, are replotted, we find that they can be divided into two groups: Al₂O₃-poor, MgO-rich dykelets, which correspond to little fractionated cumulate composition, and Al₂O₃-rich, MgOpoor dykelets, which might be the nondeformed ones; their

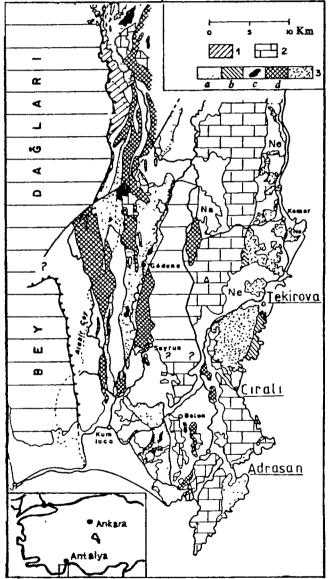


Fig. 1. Simplified structural and petrographical map of the study area, the southwestern segment of the Antalya Nappes, showing the distribution of ophiolitic facies in the median nappe and the two massifs of main interest: the tectonite massif of Adrasan and the tectonite/cumulate massif of Cirali/Tekirova. (1) Lower Antalya Nappe; (2) Upper Antalya Nappe; (3) Median Antalya Nappe, with: a, tectonites (mainly harzburgites and dunites, more or less serpentinized); b, layered ultramafic-mafic cumulates; c, non-layered gabbroic cumulates; d, pillow lavas (upper Trias); e, dyke complex of Kemer. White areas: sedimentary unit of Alakir Çay.

Ne: Neogene cover.

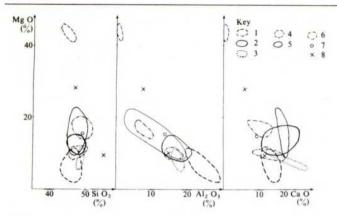


Fig. 2 Harker diagrams of bulk rock chemistry (27 analyses) showing that the differentiation from cumulate host rock—residual pockets and gabbro dykelets within cumulates—gabbro dykelets within tectonites mainly consists of a decrease in MgO and an increase in Al₂O₃. 1, Gabbro dykelets in the tectonites; 2, gabbro dykelets in the cumulates; 3, residual pockets in the cumulates; 4, tectonite host rock; 5, cumulate host rock; 6, gabbro dykelets from Oman⁸; 7, MORB primitive liquid (mean of values from refs 9-12); 8, Cyprus upper pillow lavas¹³.

emplacement might be explained in the same way as for Antalya.

Thus, if the leucocratic gabbro dykelets within the harzburgitic tectonites are residual liquids coming from the overlying magma chamber, what is the mechanism of downward injection? Their geometry, although varying from one outcrop to another, strongly suggests their setting by hydraulic fracturing (see ref. 2): (1) The end of the dykelets may be marked by small. branching and zigzagging dykelets, matching the images found by Beach 15 for experimental hydraulic fractures. (2) At the scale of a single gabbro dykelet, anastomosing dykelets and magmatic brecciation are frequent at the dyke walls (Fig. 4a). (3) Such brecciation may result in interconnected anastomosing dykelets (Fig. 4b), and even in large brecciated areas, occurring preferentially near the contact between tectonites and cumulates. As the liquid is more abundant, they are better developed on the cumulate side (Fig. 4c). (4) Straight, long, parallel dykelets have also been set by hydraulic fracturing (see below) (Fig. 4d).

Hydraulic fracturing probably occurred after the interstitial liquid had been expelled from between the cumulus minerals, since traces of such interstitial minerals are rarely observed in

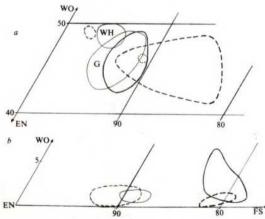


Fig. 3 Plot of clinopyroxene (a) (40 analyses) and orthopyroxene (b) (22 analyses) composition in a greatly enlarged En-Fs-Wo triangle, showing two groups neatly separated for the orthopyroxene: host rock orthopyroxene at En₉₀, and orthopyroxene of the dykelets clearly richer in Fe with En₈₀. With corresponding values the clinopyroxene show a continuous enrichment in Fe from tectonite host rock, via cumulate host rock (WH = wherlite, G = gabbro) and gabbro dykelets in the cumulates, to gabbro dykelets in the tectonites. Key as for Fig. 2.

the Antalyan cumulates. This interstitial liquid would be gathered in residual pockets, from which extending dykelets are frequently observed (Fig. 4e), indicating that liquid overpressure within the pockets provoked fracturing. Relative abundance of liquid (locally exceeding 20% of the total rock) facilitates brecciation, particularly affecting the less permeable rocks, such as the dunites at the contact between tectonites and cumulates.

The variable geometry of these gabbroic dykelets could be explained by the interference between stresses of different origin², that is, fluid pressure (p), deviatoric stress $(\Delta\sigma)$ and confining lithostatic pressure. Cracks created by hydraulic fracturing, and thus directly dependent on the fluid pressure, will be found in conditions intermediate between the following cases: (1) Deviatoric stress is zero: fluid pressure need only overcome the confining pressure and the rock coherence. As both pressures are isotropic, cracks should be created equally in all directions, unless guided by rock anisotropy, and the result will be anastomosing veins. (2) Deviatoric stress is medium: cracks will be guided by the anisotropic stress field. As the confining pressure is weak, tensile failure parallel to $\sigma_1 \times \sigma_2$ plane is

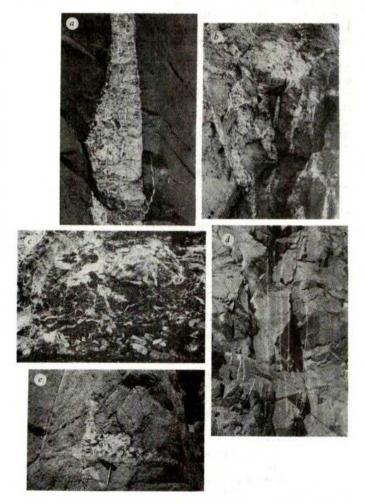


Fig. 4 a, Leucocratic gabbro dyklet (10–25 cm wide) within the tectonites of Adrasan. Foliation S2 is parallel to the dykelet and especially strong in the mylonitic shear zones. The small, branching dykelet is typical of hydraulic fracturing. b, Anastomosing gabbro dykelets, resulting in brecciation of the harzburgitic host rock, tectonite massif of Adrasan. Marker (centre) is 14 cm long. c, Brecciation at a larger scale: the 'megabreccia' of ultramafic cumulates (elements are ~1 m in diameter), cemented by gabbro. Massif of Cirali/Tekirova. d, Straight, parallel dykelets (1–5 cm) in the harzburgites of the Adrasan tectonite massif. e, A pegmatitic residual pocket with an amphibolitic core in melanocratic gabbro of the Cirali/Tekirova cumulates. Note the leucocratic dykelets extending in all four directions. Pencil (bottom centre) is 15 cm long.

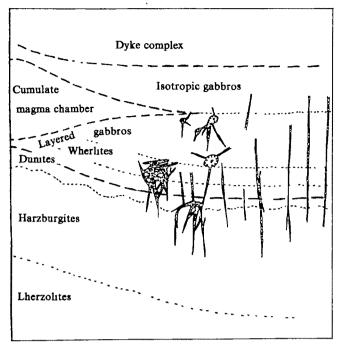


Fig. 5 Schematic model of a mid-oceanic ridge to show that the emplacement of the leucocratic gabbro dykelets by hydraulic fracturing takes place at a late stage in the evolution of the cumulate magma chamber.

possible, if the fluid pressure p yields $p > 3\sigma_2 - \sigma_1 + T_0$ (where T_0 = tensile strength¹⁶). Dykelets resulting from such tensile fracturing initiated by fluid pressure will be long, straight, and parallel to one another. (3) Deviatoric stress is high: cracks will still be guided by the anisotropic stress field, but instead of tensile fractures, shear fractures will develop. Such fractures are oblique on the $\sigma_1 \times \sigma_2$ plane, short and irregular, with a characteristic en echelon arrangement. With increasing fluid pressure the propagation speed of such cracks, and its deviation from the theoretical shear fracture direction, will increase¹⁷. The resulting more irregular fractures and especially their en echelon arrangement have also been observed in a lesser amount. They appear to be anterior to the anastomosing and then to the straight dykelets.

The stress conditions in an oceanic spreading centre clearly depend on the locality below the ridge. Thus, a given rock assemblage will be submitted to varying stress regimes as it travels away from the ridge centre. Hydraulic fracturing, although occurring at a late stage with respect to the cumulate magma chamber's evolution, must be active over a longer span of time, as several generations of dykelets produced by hydraulic fracturing, or dykelets cutting magmatic breccias are observed. At one outcrop the younger the dykelet, the more differentiated its chemistry. In general the succession of the different vein geometries points to high deviatoric stress conditions nearest to the spreading centre, passing via isotropic conditions to tensile stress away from the ridge.

In conclusion, the leucocratic gabbro dykelets cutting the tectonite and cumulate sequences of the Antalyan ophiolitic complex, constitute the latest generations of all the dykelets observed (except for the much later injected diabase dykes). They have been formed by hydraulic fracturing in variable stress conditions. They are not the feeding channels of the overlying magma chamber, which must be looked for among the other dykelets (I.R., in preparation), but their very differentiated chemistry indicates that they have originated from the overlying cumulates. They have been injected at a late stage of the cumulate magma chamber evolution in all directions in the cumulates, and downwards into the tectonites (see Fig. 5).

We thank A. Nicolas, R. G. Coleman and T. Juteau for helpful remarks and discussions. Financial support was provided by ATP 'Geodynamique' from the French INAG.

Received 26 May, accepted 18 December 1981

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Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments

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It has been generally believed that sulphate reduction precludes methane generation during diagenesis of anoxic sediments^{1,2} Because most biogenic methane formed in nature is thought to derive either from acetate cleavage or by hydrogen reduction of carbon dioxide³⁻⁶, the removal of these compounds by the energetically more efficient sulphate-reducing bacteria can impose a substrate limitation on methanogenic bacteria? However, two known species of methanogens, Methanosarcina barkeri and Methanococcus mazei, can grow on and produce methane from methanol and methylated amines¹⁰⁻¹³. In addition, these compounds stimulate methane production by bacterial enrichments from the rumen 11,14 and aquatic muds Methanol can enter anaerobic food webs through bacterial degradation of lignins¹⁵ or pectin¹⁶, and methylated amines can be produced either from decomposition of substances like choline, creatine and betaine 13,14 or by bacterial reduction of trimethylamine oxide¹⁷, a common metabolite and excretory product of marine animals. However, the relative importance of methanol and methylated amines as precursors of methane in sediments has not been previously examined. We now report that methanol and trimethylamine are important substrates for methanogenic bacteria in salt marsh sediments and that these compounds may account for the bulk of methane produced therein. Furthermore, because these compounds do not stimulate sulphate reduction, methanogenesis and sulphate reduction can operate concurrently in sulphate-containing anoxic sediments.

Sediments were taken from the upper 15 cm of a salt marsh located in San Francisco Bay at Palo Alto, California. The black, highly reduced sediments contained methane (~8 ml per 1 wet sediment; T. Vogel et al., unpublished data) and smelled strongly of hydrogen sulphide. The sediments also contained abundant plant material (such as roots, rhizomes, stems and leaves) of Spartina foliosa. Glass jars were filled completely with sediment (to exclude air) sealed and transported back to the laboratory. Experiments began within 1 h of sample collection. Sediments were homogenized anaerobically 18° with San Francisco Bay water (salinity $\simeq 20\%$; sulphate $\simeq 18$ mM) to pro-

Table 1 Effect of methanol and sulphate on formation of methane and sulphide by sediment slurries incubated for 6 days

Conditions	CH4 (µmol per flask)	S ⁻² (µmol per flask)
Methanol+sulphate	192 ± 28	126 ± 13
Methanol - sulphate	162 ± 16	104 ± 22
No amendments + sulphate	0.90 ± 0.05	111 ± 22

Results represent the mean ± 1 s.d. of three flasks. Sulphide was measured by cadmium analysis of acidified sediments on an initial levels were $\sim 9~\mu mol~S^{-2}$ per flask. The artificial media contained (g l⁻¹)· NaCl, 12; MgCl₂·6H₃O, 55; CaCl₂·2H₂O, 0.75; KCl, 0.38; NaBr, 0.04; NaHCO₃, 0.25; Na₂SO₄ or NaCl, 2.8; trace element solution on the sediment homogenate (1·1, sediment: media) Sulphide produced in the sulphate-free flasks was probably from organic sources and reduction of interstitial sulphate ions that were originally present in the sediment sample. Sulphide production was stimulated by amendment with either accetate or H₂ (S.P. and R.S.O., in preparation). Hydrogen also stimulated CH₄ production by these sediments. After 5 days, H₂-incubated slurries produced more CH₄ (8 2 \pm 5.3 μ mol per flask) than N₂-incubated slurries (0.098 \pm 0.007 μ mol per flask). However, much more H₂ was consumed by the slurries (506 \pm 61 μ mol than required for methanogenesis, because of H₂ oxidation by sulphate-reducing bacteria?

duce a dense homogenate which was dispensed into Erlenmeyer flasks and sealed under N2. In some experiments, the homogenate was further diluted with bay water to yield a slurry¹⁸. To test the influence of sulphate on methanogenesis from methanol or trimethylamine, dilute slurries were also prepared by homogenizing sediment in a sulphate-free artificial media and dispensing the homogenate into flasks containing more of the same media either amended with or lacking sulphate (Table 1). Additions were made to selected flasks, where indicated, of homogenized Spartina material, 14C-methanol and methanol. 2-bromoethanesulphonic acid (BES) was added to certain flasks to inhibit methanogenic bacteria 18,19. BES functions as a specific inhibitor of methanogens because the compound is a structural analogue of coenzyme M (2-mercaptoethanesulphonic acid)¹⁹; coenzyme M occurs only in methanogenic bacteria²⁰. All flasks were incubated in the dark at 19 °C with constant rotary shaking (200 r.p.m.).

Incubation of sediment homogenates in the presence of BES inhibited methane formation and caused increases in the pool sizes of methanol and trimethylamine with time (Fig. 1). In contrast, uninhibited flasks produced methane and had lower, fluctuating pool sizes of both methanol and trimethylamine. Hydrogen did not accumulate in appreciable quantities (~100–200 nmol per flask; day 2) in either the BES or the uninhibited flasks, and the gas was not detected by day 3 in any of the flasks. In a second experiment, addition of ¹⁴C-methanol to freshly prepared sediment homogenates resulted in the immediate, linear production of ¹⁴CH₄ and production of both ¹⁴CH₄ and CH₄ were inhibited by BES (Fig. 2).

In experiments with dilute sediment slurries, methanolamended flasks (500 μ mol per flask) produced methane and production levelled off after 5 days incubation. Levels of methane formed in replicate flasks (310 and 340 μ mol per flask) accounted for an 83–91% conversion of the methanol, based on the reaction:

$4CH₃OH \rightarrow 3CH₄ + CO₂ + 2H₂O$

Flasks incubated with methanol plus BES (3.5 mM) did not produce significant amounts of methane ($<0.5 \,\mu$ mol CH₄ per flask after 2 weeks incubation). Methane formation (from methanol) by sediment slurries incubated in artificial media was not influenced by the presence of sulphate. The rates and extent of methane formation with time were identical for flasks which either contained or lacked sulphate ions (20 mM) and the levels of methane achieved at the end of the experiment (6 days) were comparable (Table 1). Amendment of these slurries with methanol greatly stimulated methanogenesis but did not stimulate sulphate reduction (as measured by S⁻²; Table 1). We have obtained similar results for trimethylamine using slurries pre-

pared from nearby intertidal mudflat sediments (S.P. and R.S.O. in preparation).

The accumulation of trimethylamine and methanol in BESinhibited homogenates (Fig. 1) indicates that these compounds are usually present in these sediments, but are continuously removed and converted to methane by methanogenic bacteria. In addition, no lag of ¹⁴CH₂ production occurred on addition of ¹⁴C-methanol to sediment homogenates (Fig. 2). Because production of ¹⁴CH₄ was immediate and linear, the methanogens present in the sediments were substrate adapted to methanol and presumably, trimethylamine (14C-trimethylamine was not tested). Furthermore, because 4 mol of trimethylamine can produce 9 mol of methane¹³, the large pool sizes of trimethylamine (Fig. 1b) at day 2 (~57 µmol per flask, corresponding to $\sim 570 \, \mu \text{mol l}^{-1}$) could account for all the methane produced (~37 µmol CH₄ per flask at day 3) with 10% coming from methanol. Hydrogen was not an important energy source for methanogens because there was no appreciable accumulation of this gas either in the presence or absence of BES. Disappearance of hydrogen by day 3 therefore, was due to removal by sulphate reducers and other anaerobic bacteria⁷

As methanol and trimethylamine stimulate methanogenesis, but not sulphate reduction, the two processes can operate simultaneously and independently within anoxic sediments containing these compounds and sulphate ions. It is significant that other workers have observed a rapid conversion of ¹⁴C-methylamine to ¹⁴C-methane in sulphate-containing marine sediments (M. Winfrey and D. Ward, personal communication). Thus, in sediment pore waters containing relatively high concentrations of sulphate, sulphate reduction proceeds at the expense of short chain fatty acids and hydrogen⁹ while methanogenesis can occur simultaneously through metabolism of non-competitive substrates, such as methanol and methylated amines. As sulphate becomes depleted from sediment pore waters, short chain fatty acids and hydrogen can enter

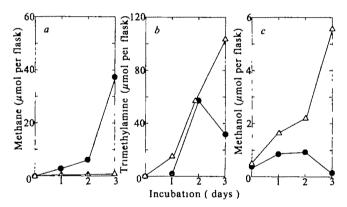


Fig. 1 Levels of methane (a), trimethylamine (b) and methanol (c) in uninhibited flasks (●) and flasks containing 0.7 g BES (△) Flasks (total volume, 138 ml) contained 80 ml sediment homogenate (1.1, sediment bay water), 10 ml bay water and 20 ml homogenized S. foliosa materials (1.1 with bay water) A set of eight flasks (four uninhibited, four + BES) were incubated with constant shaking (dark, 19 °C) Two flasks were used daily for methanol and trimethylamine determinations. Homogenate was centrifuged (4°C) and supernatant distilled (90-100°C) Fractions of distillate (100- $500\,\mu l)$ were collected and analysed by flame ionization gas chromatography 18 using 5 μl injections and a 183×0 64 cm stainless-steel Porapak Q column (110 °C) Compounds were identified by retention time. Per cent recoveries of methanol (75%) and trimethylamine (46%) were obtained by distillation of standard solutions, however, distillation efficiencies were not applied to pool size estimates for the purpose of being conservative. The presence of trimethylamine in the distillate was confirmed by mass spectrometry (Accurex Co) One µl of sample was purged onto a Tekman LSC 2 and thermally absorbed onto 0 5% carbopak 1500 carbopak column. The column was ramped from 60 to 200 °C and effluent detected on a Finnegan 1020 gas chromatograph-mass spectrometer scanning 41-275 AMU every 2 s The samples had AMU peaks at 59 (trimethylamine) and were identical with prepared standards. Methanol also accumulated in flasks that contained only homogenized sediments (no added plant materials) After 7 days incubation, 5.5 and 332 nmol CH₃OH were recovered from uninhibited and BES flasks, respectively (trimethylamine was not searched for) The flasks had produced 3,200 nmol CH₄ (unmhibited) and 300 nmol CH₄ (BES) after 1 week

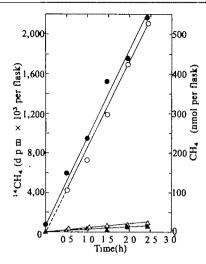


Fig. 2 Formation of CH₄ and ¹⁴CH₄ by homogenates amended with ¹⁴C-methanol (12.5 μCi per flask; 0.28 μmol per flask, ICN Co, initial specific activity 45 mCi mmol⁻¹) Unhibited flasks: ¹⁴CH₄, Φ; CH₄, O. Flasks inhibited with BES (1 g). ¹⁴CH₄, Δ, CH₄, Δ Flasks (total volume, 263 ml) contained 145 ml of sediment homogenate (1.1 with bay water), 25 ml bay water and 65 ml of homogenized S foliosa materials (1.1 with bay water) Flasks were incubated in the dark (20°C) with constant rotary shaking (200 r.p.m.) ¹⁴CH₄ and ¹⁴CO₂ were determined by gas chromatography—gas proportional counting procedures²⁷ and CH₄ by gas chromatography¹⁸

methanogenic pathways. Therefore, the ecological niche occupied by methanogens seems broader than previously predicted^{1,2}. As most studies on sediment methanogenesis used ¹⁴Cacetate or ¹⁴C-bicarbonate as precursors of methane^{6,21}, results can be misleading with regard to zones of methane production and may also underestimate carbon budgets. Future studies should be directed at determining the contribution methanol and methylated amines make to the methane formed in various aquatic sediments. Because carbon isotope enrichment factors are significantly greater in methanol-grown methanogens (~70%)^{23,26} than in hydrogen plus carbon dioxide-grown cells (~40%)²², such information would be of importance for the interpretation of carbon isotopic fractionations of methane during methanogenesis in anoxic sediments.

We thank R. Gunsalus, D. Ward and M. Winfrey for helpful discussions, K. Kvenvolden, R. Smith and W. Balch for discussions and manuscript review, and G. Nicoll for the mass spectrometry work. Mention of brand name products does not constitute an endorsement by the US Geological Survey.

Received 22 October, accepted 18 December 1981

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Sulphate and sulphate reduction in early Precambrian oceans

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Sulphate reduction generally causes isotopic fractionation of sulphur¹. Modern sedimentary sulphide is largely produced by biogenic reduction of sulphate and is typically enriched in ³²S (ref. 2). This is balanced by excess ³⁴S in the oceanic sulphate reservoir and evaporites3. High-temperature, inorganic reduction of sulphate may also cause fractionation^{4,5}. Since the work of Ault and Kulp⁶, there has been interest in finding the beginnings of sulphate reduction in the sedimentary record. This is important for several reasons. First, sulphate-respiring bacteria are a milestone of evolution^{7,8}. Second, it established the exogenic sulphur cycle in an essentially modern form. This, with the interconnected oxygen and carbon cycles, regulates the composition of atmosphere and oceans⁹⁻¹¹. Third, widespread evidence of sulphate reduction in rocks of a given age and younger indicates that sulphate was established as a major constituent of seawater. In addition to identifying a stage in the evolution of an oxygenated environment¹⁰, this has important metallogenic implications. Schidlowski⁸ has recently concluded that dissimilatory reduction commenced at 2.800-3,100 Myr in an Archaean ocean that had relatively high concentrations of sulphate. I review here the published data and present additional sulphur isotope analyses obtained from the early Precambrian of South Africa. These results indicate that sulphate was a minor component of Archaean and early Proterozoic ocean water, probably <0.001 mol l⁻¹. The concentration had increased by ~2,350 Myr to levels allowing significant biogenic and inorganic fractionation and the partitioning of ³²S/³⁴S in the exogene cycle.

Information provided by Precambrian evaporitic sediments is limited. The oldest Proterozoic evaporites for which data are published have maximum ages of only 1,300 Myr. These have 834S values of up to 30% (refs 12,13), indicating that sulphate reduction was well established at that time. Stratiform barites found in >3,200 Myr rocks on several continents have δ^{34} S of ~0\% indicating no significant isotopic partitioning in the oceans of that time.

Figure 1 gives the means and standard deviations of δ^{34} S for groups of sedimentary sulphide of Archaean (>2,500 Myr) and Aphebian (2,500-1,800 Myr) age. Where geological information permits, the latter have been subdivided into 'Aphebian 1' (>2,300 Myr) and 'Aphebian 2' (≥2,300 Myr). Data have been excluded where the rocks may have been affected by metasomatism. Care must be taken in interpreting the statistical data, as the sample collections vary greatly in size and in stratigraphical and geographical extent.

Several generalized fields have been outlined in Fig. 1, related to the genesis of the sulphides. Magmatic sulphides have δ^{34} S values close to 0% and low variance. Groups with more positive δ^{34} S values, but also low variance, have been identified as the product of partial reduction of ocean sulphate at high temperature, for example, the Red Sea¹⁴ and in Homestake Mine sediments¹⁵. However, depending on the degree of fractionation⁵ and δ^{34} S of ocean sulphate, this field can presumably extend into or across the 'magmatic' field.

Sample groups having distinctly negative $\delta^{34}S$ values and moderate to high variance are attributed to biogenic sulphate reduction in systems open to exchange with ocean sulphate¹⁶. Groups enriched in ³⁴S and with moderate to high variance have been imputed to closed-system sulphate reduction 17,18 that presumably may be either inorganic or biogenic.

All of the Archaean and 'Aphebian 1' groups fall into a restricted range close to 0% $\delta^{34}S$ and low variance, characteristic of magmatic sulphur. The possible exceptions are the 2,750 Myr Woman River and Michipicoten iron formations. The greater variation found in these two groups has been ascribed to biological reduction of low concentrations of sulphate within a restricted basin¹⁹. This interpretation has not been universally accepted²⁰⁻²². As the metals of the iron formations were almost certainly derived from thermal springs²³, fractionation of sulphur at high temperature may have been involved. Indeed, sulphide formed by high-temperature reduction will tend to show the greatest variation in $\delta^{34}S$ at low sulphate concentrations, that is $\Sigma SO_4^2 = \Sigma H_2S$, because of the sensitivity of fractionation to solution chemistry near the SO_4^2 / H_2S boundary⁵.

While the evidence for bacterial reduction in the Archaean is debatable, there is little doubt that during this time there was no quantitatively important partitioning of ³²S/³⁴S in the exogene cycle, as shown by the data from five continents summarized in Fig. 1 that have means clustered near 0%. Also, on the basis of the limited published evidence, this condition persisted into 'Aphebian 1' time.

Partitioning was established at the time of deposition of the Karelian schists²⁴ at ~2,200 Myr. Also, the Outokumpu deposit, a submarine exhalative massive sulphide body within the Karelian, dated at 2,250 Myr (ref. 25), shows a wide range of δ^{34} S from -19.2% to 5.8% (ref. 26). Unfortunately, some of the most comprehensive data on sedimentary sulphides of Aphebian age come from strata whose age is known only within broad limits, that is at the Homestake Mine¹⁵ and the Pine Creek geosyncline²⁷. The enrichment of ³⁴S in many of the early Precambrian units measured to date is intriguing and requires to be balanced by excess ³²S elsewhere²⁸.

To provide further data on sulphur isotope fractionation, I have sampled shaly sediments in the Republic of South Africa, representative of the period 3,300–2,000 Myr. This country contains some of the most complete and best exposed early Precambrian successions. Moreover, cratonic conditions were established at a relatively early date here^{29,30}, giving rise to Archaean sediments of different facies from the more typical 'greenstone belt' type. This is important, as the volcanogenic sulphide which dominates the greenstone belts²⁸ may have obscured any biogenic contribution.

Because sulphide is often difficult to separate physically from carbonaceous shale, the sulphur isotopic data were mainly obtained by burning powdered shale in O_2 at 1,300 °C to produce SO_2 . Analyses of pyrite and/or pyrrhotite separated from 20 of these samples give similar $\delta^{34}S$ values to the total

burn. The organic carbon and the sulphur contents are quite variable for the samples from the Swaziland and Transvaal Supergroups, with values to 5.5% C and 3.2% S for the former and 9.6% C and 4.5% S for the latter. The West Rand Group samples have lower values for carbon, ranging to 0.4% C and 4.2% S.

Deposition of the Transvaal sediments took place mainly in a shallow, marine environment³¹, while the upper part of the Onverwacht was laid down in shallow water, in part possibly evaporitic³². The depositional site of Witswatersrand strata has been enigmatic, either a shallow inland sea, or a lake³³. But Watchorn³⁴ found tidal features in the West Rand Group and both fluvial and shallow water marine facies. Many of the West Rand shales are low grade iron formations, which has been taken as evidence of marine origin³⁰.

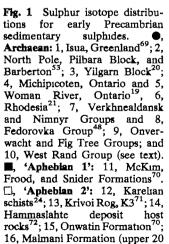
The results (Fig. 2) show δ^{34} S values close to 0% for all

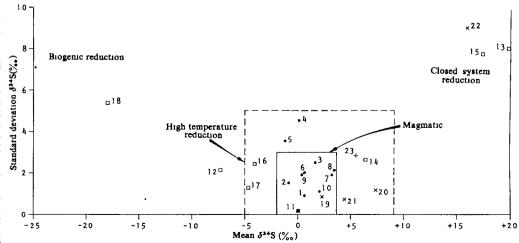
The results (Fig. 2) show δ^{34} S values close to 0% for all samples up to and including the lowermost part of the Malmani Subgroup. This reflects a magmatic source of the sulphide, either directly from volcanic exhalations, or from detrital sulphide that survived weathering in an O_2 -poor atmosphere. Above this, the character of the isotopic distribution is entirely different, with mainly negative numbers. I suggest that the sulphides from the shales above the isotopic transition were derived from reduction of sulphate, with both biogenic and inorganic processes probably being involved.

Sulphide from carbonaceous shale of the Timeball Hill Formation is undoubtably biogenic with whole rock values to -30.6% (pyrite from the same sample measured -30.5%). Two sections were sampled, one being the basal 30 m of the formation in a drill core at Mafefe, northern Transvaal and the other the basal 10 m from a core at Fochville, 400 km distant. The lowest ³⁴S values are from the most sulphidic shales.

Two 20-m sections of pyritic shale from the basal Malips Member of the Penge Iron Formation were taken: one from Mafefe, the other at Penge, 45 km away. They show very similar δ^{34} S values with means of -4.8% and -4.9% respectively and a low standard deviation for all samples of 1.3%. These absolute values, plus the uniformity of the data, suggest derivation by high-temperature reduction, then thorough mixing before or after exhalation.

The Malmani samples are from carbonaceous shale interbeds in this carbonate unit and are taken from a 1,200-m section near Fochville, plus two samples from Mafefe immediately below the Penge Formation. In part, these data may be interpreted (Fig. 2) as hydrothermal sulphide, similar to the Malips Member. The Malmani is a chemical precursor of the great iron deposits of the Penge and Kuruman Formations, with cycles of iron enrichment in the carbonate³⁵. However, caution





samples, Fig. 2), 17 Penge Formation; 18, Timeball Hill Formation (see text). ×, Aphebian undivided: 19, Cahill Formation²⁷; 20 Homestake Formation; 21, Poorman Formation and 22, Ellison Formation¹⁵. +, Modern: 23, Red Sea, hydrothermal (SU1 and SU2 zones)¹⁴. Additional geochronological data from ref. 73.

must be used in interpreting these data, as both upper intertidal and subtidal shales have been identified in the Malmani (ref. 36 and N. J. Beukes personal communication). Each shale type is likely to have a different isotopic distribution. The isotopic transition (Fig. 2) is placed between samples 45 and 77 m above the base of the Malmani. The samples above and below the transition, including those from the Black Reef, are similar, being carbonaceous to highly carbonaceous shales.

The Malmani is bracketed by age determinations of 2,640 Myr for the middle group of the Ventersdorp³⁷ and 2,240 Myr for the Ongeluk lavas of the Pretoria Group (D. Crampton, in ref. 38). Both of these horizons are separated from the Malmani by major unconformities. However, the base of the Malmani is probably closer in age to the Ongeluk than to the Ventersdorp. For this reason the isotopic change is estimated at ~2,350 Myr, a figure likely to be changed with more detailed geochronology.

The isotopic transition at $\sim 2,350$ Myr is indicative of one of two possible conditions, the first being an increase in the sulphate concentration of seawater to a level sufficient for reduction to cause significant isotopic fractionation. Harrison and Thode³⁹ showed that the fractionation by Desulphovibrio desulphuricans is sharply reduced at $< 0.001 \text{ mol}^{-1} \text{ SO}_4^{2-}$, equivalent to ~4% of the concentration in present seawater. For inorganic reduction over the temperature range 200-350 °C at neutral pH, fractionation is minimal when $\Sigma SO_4^{2-} < \Sigma H_2 S$ (ref. 5).

The second possible explanation is that sulphate-reducing bacteria evolved at ~2,350 Myr in a pre-existing, sulphate-rich ocean. This is less plausible if it is accepted that the Aphebian also marks the first significant appearance of sedimentary sulphide produced by high-temperature reduction of sulphate, for example, the Homestake Formation¹⁵ and the data given

here.

Further evidence of an evolutionary change from a lowsulphate Archaean ocean is the sulphur isotope composition of massive sulphide base metal deposits. Those of Proterozoic and younger age have mean δ^{34} S values that commonly differ significantly from 0% (refs 40,41), a fact which has been ascribed to derivation, in part at least, by reduction of seawater sulphate 40,42,43. Late Archaean (~2,700 Myr) massive sulphides have mean values close to 0% (ref. 41), although seawater also had a major role in their formation⁴⁴. Sulphate minerals are absent in these Archaean deposits^{45,46}, whereas they are relatively common in younger deposits.

Possibly contrary evidence is the occurrence of stratiform barite in the Archaean of southern Africa^{32,47-51}; in the Pilbara Block of western Australia⁵²⁻⁵⁴; in India⁵⁵; and in the USSR⁴⁸. Where reliable age determinations are available, it seems that these sulphates occur in older Archaean strata, that is

 $>3,200 \, \text{Myr}.$

Studies carried out on the little metamorphosed Pilbara and Barberton occurrences indicate a depositional environment unusual for the Archaean. That is, a rather extensive, tectonically stable shallow-water shelf with volcanic vents but little detritus⁵⁴. At least some sulphate was deposited in evaporitic conditions as gypsum, then replaced by silica and barite 53,54,56 Younger Archaean strata, such as that which dominates the Canadian Shield, lack barite⁵⁰ and shallow water environments of the type described above are rare.

The Pilbara and Barberton barites probably were deposited in restricted basins where sulphate produced, for example, by bacterial oxidation of sulphide⁴⁷, could increase to higher concentration than in the ocean. Mean δ^{34} S values of 3.6-3.8% for these barites⁵³ indicates that isotopic partitioning was not established in the early ocean. The model discussed below linking global tectonics with ocean sulphate content does not, however, preclude short-term increase in sulphate within Archaean oceans in response to any temporary decline in rates of plate formation.

The data presented here indicate that during Archaean time sulphate was a minor constituent of seawater, probably <0.001 mol l⁻¹, except within restricted basins. This condition

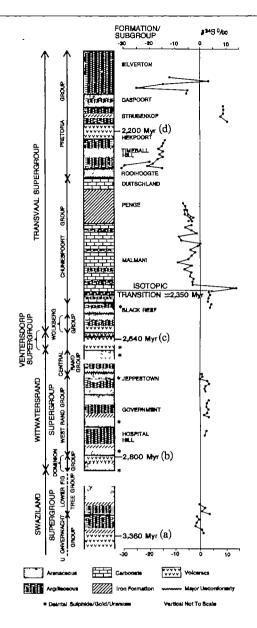


Fig. 2 Sulphur isotope results, early Precambrian argillaceous sediments, Republic of South Africa. Stratigraphical thicknesses not to scale. Isotopic data plotted in true stratigraphical order but not to represent a stratigraphical position within any given unit. Geochronological data: (a) ref. 74; (b) ref. 75; (c) ref. 37; (d) D. Crampton in ref. 38.

persisted into the Proterozoic, but by ~2,350 Myr, sulphate had reached a level sufficient for biogenic or inorganic reduction to cause significant isotopic fractionation and the partitioning of ³²S/³⁴S in the exogene cycle.

The change in the sulphate concentration of the ocean coincides with a suggested initial increase in the oxygen content of the atmosphere above the primitive level of $<(10^{-2}-10^{-6})$ PAL required for the deposition of placer uraninite⁵⁷. The isotopic transition (Fig. 2) occurs immediately above the youngest occurrence in South Africa of detrital sulphides and uraninite in the Black Reef^{58,59}. In the Huronian of Canada a change from strata hosting pyritic-uraniferous conglomerates to those with black (oxide) sands and red beds occur at ~2,300 Myr (ref. 60).

The transition, during the Lower Proterozoic, from an anoxic atmosphere to one with low levels of O₂ has been attributed to the filling of oceanic sinks for O_2 , notably Fe^{2+} derived from continental weathering ⁶¹⁻⁶⁴. It is curious that this transition and the increase in oceanic sulphate discussed here, approximately coincided with the greatest tectonic milestone in geological history: the change from a highly mobile Archaean crust to a Proterozoic regime of stable continental masses. Consideration must be given to alternative models, relating atmospheric and hydrospheric evolution to tectonic change.

The dominant mechanism for maintaining O₂ and SO₄² at low levels during the Archaean may have been a greater rate of exchange of reduced materials between the mantle and the ocean. Edmond et al.65 estimate the flux of SO₄ into modern submarine geothermal systems as equivalent to that entering the oceans from rivers. Moreover, major amounts of Fe2+ and Mn²⁺ are introduced into the oceans from the ridges.

As the driving force for the exchange between mantle and ocean is heat flow, one would expect this to be greater in the Archaean. Furthermore, there is reason to believe that the exchange was even greater in the Archaean than might be predicted from a linear relationship with the Earth's heat flow. Burke and Kidd⁶⁶ suggest that during this period a greater proportion of the Earth's heat was dissipated at ocean ridges. Bickle⁶⁷ calculated that plate formation was about six times greater at 2,800 Myr than at present, compared with a factor of about three for heat flow. The Archaean/Proterozoic boundary seems to mark a change from a regime of rapid plate formation to a slower one 68 that would, in turn, produce a lower rate of exchange between reduced mantle material and the hydrosphere/atmosphere. I suggest that it is this change that allowed SO₄²⁻ to become a major component of ocean water and the entry of significant amounts of free oxygen into the atmosphere.

I thank many South African geologists for cooperation and hospitality, in particular, Professor C. R. Anhaeusser, Professor D. A. Pretorius, Dr N. J. Beukes, Professor A. O. Fuller, Dr G. L. Coetzee, P. A. Harrison, Dr W. E. L. Minter, Dr T. G. Molyneux, P. R. Strydom, J. L. Matthysen, P. J. Schoeman, J. P. van Zyl, K. J. Barnard, T. Hopkins, M. Wuth, J. Wigand, P. Becker, L. N. J. Engelbrecht, and Dr P. J. Smit. The isotopic analyses were done by Dr R. Reesman. I thank the Geological Survey of Canada for support, and Professor R. M. Garrels for stimulating discussion on the geochemistry of sulphur.

Received 12 October 1981, accepted 11 January 1982

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Pheromones in mice: reciprocal interaction between the nose and brain

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Blockade of pregnancy by odours from strange males¹⁻³ has interested considerably those that adopt a sociobiological approach to reproduction⁴⁻⁶. It has been suggested that the mechanism has evolved to promote heterogeneity in the population, and that strange males in possessing the capacity to block pregnancy thereby increase their reproductive potential. However, such knowledge as we have of the territorial behaviour and social organization of mice makes this explanation less likely as resident males have such an advantage over intruders that access by strange males is probably an infrequent event. Another explanation relates pregnancy block to the effect that the male pheromones have on the female reproductive hormones in other contexts. Male pheromones can stimulate both early puberty and induction of oestrus 10-12 in grouped females by suppressing prolactin secretion. Such a response, highly appropriate in this context, would be extremely disadvantageous following fertilization, since lowering prolactin is known to prevent implantation^{13,14}. Thus, some mechanism must exist to offset the more general effect of the male's own pheromone on the endocrine function of his female at such times, and this, we suggest, is prevented by the noradrenergic mechanism which we describe here.

In the first experiment, female mice (BALB/c), which had been housed alone for part of the experiment, were taken from their home cage and placed with BALB/c stud males for 20 min to allow mating. They were then removed to cages containing F₁ male bedding while the stud male was returned to the females' home cage. The F₁ males were derived from crossing C57 females with CBA males, and produce pheromones that

Table 1 Effect of replacing stud male odours immediately after mating on stud males' ability to block premancy

Stud male	Exposure to soiled bedding (0-48 h)	Exposure to male (48-72 h)	% Showing pregnancy block (n)
BALB/c BALB/c BALB/c BALB/c BALB/c	F ₁ BALB/c BALB/c F ₁ F ₁	BALB/c BALB/c F ₁ F	75 (8) — 0 (8) — 80 (10) — P < 0.01 — NS*

^{*} The data were subjected to χ^2 test of significance NS, not significant.

are sufficiently different from those of BALB/c males to prevent pregnancy occurring in BALB/c females. After 48 h the females were returned to their home cages containing the stud males. The control group consisted of females that were treated in the same way, except that, after mating, they were moved to cages containing BALB/c male bedding. Vaginal smears were taken daily to determine the onset of oestrus and 6 days after mating, females were killed and their uteri examined for implantation sites to demonstrate whether an olfactory block to pregnancy had occurred. The control females, which had been returned to the stud male after previous exposure to bedding from the same strain (BALB/c) showed implantation, as expected (Table 1). However, in those females exposed to F₁ male bedding for 48 h after mating, the stud male appeared to have been transformed to a 'strange' one, for subsequent exposure of the females to the stud male prevented implantation of his own fertilized

Neither exposure to F_1 bedding by itself for 48 h immediately after BALB/c mating nor a return to F_1 males after this procedure had any blocking effect on pregnancy. This experiment demonstrates that the odour cues which follow mating are the ones 'recognized' by the female's neuroendocrine system, and that this recognition is necessary for pregnancy block to occur. Stud males can thus cause fetal resorption, providing the female has received prolonged exposure to the odour of a strange male, replacing that of the stud.

How does the act of mating and exposure to odour immediately afterwards 'print' recognition of the stud male, thereby preventing him from blocking his own pregnancy? It is known that the so-called primer effects of such pheromones are transmitted by the vomeronasal accessory part of the olfactory system^{9,12,14-16}, which projects to the cortico-medial amygdala¹⁷ part of the brain closely related to the ventromedial hypothalamus, itself concerned with neuroendocrine activity. Both the accessory and main olfactory bulbs receive a noradrenergic projection¹⁸, and this system has been implicated in certain forms of recognition, for example the modulation of neural activity after exposure to certain types of visual stimuli early in life¹⁹, and the neuroendocrine response of the female rat to tactile stimuli inducing lordosis and pseudopregnancy²⁰.

To determine whether the centrifugal noradrenergic projection to the olfactory bulbs was involved in the response of the female mice to the males' pheromones, discrete lesions were made by stereotaxically injecting the neurotoxin 6-hydroxydopamine (2 µg 6-OHDA in 0.5 µl saline containing 2 µg ml⁻¹ ascorbic acid for 5 min) bilaterally into the medial olfactory

stria which carry the noradrenergic input to the olfactory and accessory olfactory bulbs. A second group of mice were injected in the same way with vehicle alone, a third group with neurotoxin directly into the accessory olfactory bulb, and a fourth group received no injections. After at least 4 days, females from each group were housed in pairs with a BALB/c stud male, which was removed the morning after mating had occurred (as assessed by observing vaginal plugs in the female); 24 h later the stud male was returned to the fertilized female and housed with her for 48 h. A fifth control group received 6-OHDA lesions to the medial olfactory stria but were kept away from all males after mating to determine whether the procedure itself interfered with pregnancy. Vaginal smears were taken daily, all females were killed 6 days after mating and the uterus examined for implantation sites; the olfactory and accessory olfactory bulbs were removed and their noradrenaline content measured.

Both groups 1 and 2 (female mice in which noradrenaline had been depleted in the accessory olfactory bulbs) failed to 'recognize' the stud male, and thus pregnancy was blocked by exposure to him, whereas the neurotoxin lesion itself (group 5) did not interfere with pregnancy (Table 2). As expected, in mice undergoing either sham operations or no treatment (groups 3 and 4), the stud male failed to block pregnancy. Measurement of the noradrenaline content showed that 6-OHDA lesions of the medial olfactory stria produced a significant decrease in noradrenaline (70%) in the olfactory and the accessory olfactory bulbs, without significantly altering dopamine (Table 2). Direct measurement of noradrenaline content in the accessory olfactory bulb was not possible because of its small size.

These experiments show that, in contrast to what is generally believed, a special neuromechanism exists not so much to ensure that strange males will block pregnancy in female mice, but so that familiar (that is, stud) males will not do so. This implies that some neural protective mechanism must be set in motion during mating which ensures that continued presence of the stud male or his odour after coitus will not prevent subsequent implantation. Such a process is dependent on olfactory input to the female which outlasts the act of coitus itself, as we have shown that it can be overridden by a second olfactory stimulus applied immediately after mating has occurred. For the neural mechanism to be effective, there must exist an intact noradrenergic input to the accessory olfactory bulb. The action of this noradrenergic mechanism in the olfactory system may be similar to that of other sensory systems, serving to enhance recognition of the stud male by increasing the signal-to-noise i1,22. Alternatively, it has been suggested that noradrenergic mechanisms may have an important role in the memory of events which have survival value²³, a 'print-now' mechanism for incoming sensory information to be stored for later retrieval¹⁹.

The response to male pheromones in female mice has selective advantages for accelerating puberty and induction of oestrus^{7,8}, but carries a risk for pregnancy should contact with these pheromones occur during a 3-day period after mating. The noradrenergic mechanism apparently sets in motion a relatively short-term neuroendocrine memory, which protects the female from pregnancy block which would otherwise be induced by her stud male.

Table 2 Effect of olfactory noradrenergic lesions in the female mouse on the stud males' ability to block pregnancy

Group	Treatment	•	% Showing pregnancy block (n)
1 2 3 4 5	6-OHDA (medial olfactory stria) 6-OHDA (accessory olfactory bulb) Sham lesion No treatment 6-OHDA (medial olfactory stria)	Re-exposure to stud male No male exposure	88 (8) 100 (8) - NS 12 (8) - P < 0.01* 0 (8) - NS

Mean noradrenaline content of olfactory bulbs in groups 3 and 1 was 0.37 ± 0.08 and 0.11 ± 0.07 ng per mg tissue, respectively, t=6.59; P<0.0004 Mean noradrenaline depletion = 70% Dopamine content of olfactory bulbs in groups 1 and 3 was 0.58 ± 0.2 and 0.38 ± 0.06 ng per mg tissue, respectively (t=0.82, not significant).

^{*} The data were subjected to χ^2 tests of significance.

We thank Anne Lloyd-Thomas, Barry Everitt, Gerald Moore and Joe Herbert for thought-provoking discussion.

Received 9 November 1981; accepted 29 January 1982.

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Extensive elongation of axons from rat brain into peripheral nerve grafts

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The failure of axons to elongate in the injured central nervous system (CNS) of adult mammals restricts drastically the establishment of connections with target tissues situated more than a few millimetres away. Mechanisms that include a primary inability of some nerve cells to support renewed axonal growth, a premature formation of synapses on nearby neurones¹, an obstruction caused by the formation of a glial scar^{2,3} and other influences of the microenvironment4-7 are presumed to contribute to the failure of nerve fibres to regenerate as effectively in the CNS as in the peripheral nervous system (PNS). Support for the hypothesis that conditions in the glial environment of injured fibres have a decisive role in successful axonal elongation has recently come from studies using transplants containing either central glia or peripheral nerve segments as conduits of axon growth^{7,8}. While CNS glial grafts have been shown to prevent growth of PNS fibres^{7–9}, experiments which used labelling techniques to trace the source of axons growing into PNS grafts provided evidence that processes from nerve cells in the spinal cord and medulla oblongata of adult rats may increase in length by 1 or more centimetres when the CNS glial environment is replaced by that of peripheral nerves 10,11. Here we report for the first time the extensive elongation of axons from neurones in the brain of adult rats through PNS grafts introduced into the cerebral hemispheres.

In 20 Sprague-Dawley rats weighing ~300 g the end of a 15-mm segment of an autologous sciatic nerve was inserted close to the basal ganglia or to the cerebral cortex (Fig. 1A). The animals had previously been anaesthetized with sodium pentobarbital administered intraperitoneally (5 mg per 100 g body weight). A glass rod held in a micromanipulator was inserted through an opening in the frontal bone and dura to a depth calculated to produce lesions extending to the sensory cortex or basal ganglia. The sciatic nerve graft was then introduced into each of these lesions and its epineurium anchored to the dura mater with 10-0 nylon sutures. The space between the nerve and the surrounding skull was sealed with bone wax and the free outer end of the nerve sutured to the temporalis muscle. The animals survived without any apparent neurological deficit other than limb weakness resulting from the removal of one sciatic nerve. The rats were examined 5-23 weeks after grafting to determine whether axons from neurones within the brain had elongated along the graft. For this purpose the graft was dissected along its extracranial course, transected 2 mm from its insertion into the temporalis muscle, and the free end placed for 50 min in a pad of Gelfoam soaked in a 20% solution of horseradish peroxidase (HRP; type VI, Sigma). The remaining portion of the graft was placed over a plastic sheath and covered with vaseline to avoid contamination of the surrounding tissue by HRP (Fig. 1A). In five additional animals, used as controls, the mid-portion of the regenerated graft was crushed three times with fine-tip forceps cooled in liquid nitrogen, 9-16 weeks after grafting and half an hour before the application of HRP to the distal end of the crushed nerve. About 48 h after applying HRP, the animals were perfused through the heart with a 0.1 M phosphate buffer solution followed by 3% glutaraldehyde. The brain was removed and rinsed in 10% sucrose buffer overnight. Then, 40 µm-thick coronal sections were cut in a cryostat, reacted with tetramethylbenzidine and hydrogen peroxide¹², counterstained with neutral red and mounted in Permount.

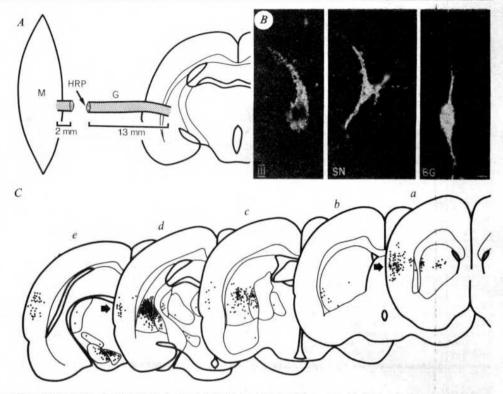
Cross-sections of the outermost portion of each graft were examined using a light microscope and found to contain regenerated fibres surrounded by Schwann cells and myelin. The overall appearance of these fibres resembled that of any regenerated peripheral nerve. HRP-labelled neurones were sought by dark- and brightfield light microscopy in sections rostral to and including the midbrain. An average of 145 sections of the brain were examined per rat. In 10 experimental animals the graft terminated close to the cortex; in 8 it was found within the caudate putamen and in 1 rat each it reached the hippocampus or the ventral nucleus of the thalamus. Of a total of 444 HRP-labelled neurones (Fig. 1B, C), most were found in the caudate putamen and sensory cortex (Table 1). The labelled nerve cells in the sensory cortex were scattered through layers II-VI. Both large and small cortical neurones were labelled but the HRP-containing cells tended to be of larger size than the unlabelled ones in the same regions (Fig. 2). Most (79%) of the labelled cells found in the brain of these animals were within 1.5 mm of the intracerebral tip of the graft; the rest were located between 1.5 and 6.5 mm from the graft. The greatest distance between the graft and the soma of the labelled cells was found for neurones in the substantia nigra (SN). Because all labelled neurones in the experimental rats were confined to a well circumscribed area close to the graft and as only two neurones were labelled in the five controls in which the graft was crushed before HRP application, we believe that most of the cells in the experimental animals used in this study were not falsely labelled by haematogenous spread or by diffusion along the grafted nerve. Thus, on the basis of these HRP tracing studies, we conclude that axons of cells in different regions of the rat brains examined have become elongated by at least 13 mm, the length of nerve graft used in the labelling experiments.

The fact that the largest number of labelled neurones was found in the caudate putamen may reflect a greater ability of

Table Distribution of HRP-labelled cortical and subcortical neurones in the brain of rats having PNS grafts

Site	No. of HRP- labelled neurones
Caudate putamen	218
Globus pallidus	42
Thalamus	10
Substantia nigra	22
Ventral tegmental area	20
Zona incerta	4
Amygdala	1
Claustrum	1
Cortex	126
Total	444

Fig. 1 A, Schematic representation of the experimental design: a PNS graft (G) has been introduced into the rat brain. The intracranial tip of the graft can be positioned close to the cortex or subcortical nuclei within the hemisphere. The outer end of the graft, initially attached to the temporalis muscle (M), is cut to apply HRP. The advantages of this design are: (1) as most of the graft is extracranial, the cells from which the axons growing into the graft have originated can determined by retrograde labelling with little risk contamination from spread of HRP into the brain; (2) the length of axons regenerating within the graft can be measured by determining the distance between the site of HRP application and the soma of the labelled cells; (3) the grafts may be positioned in several regions of the brain to test the regenerative capacity of different neuronal populations. B, darkfield light micrographs of HRPlabelled neurones in layer III of the sensory cortex, from the pars compacta of the substantia nigra (SN) and from basal ganglia (BG).



Calibration bar, $10 \mu m$. C, transverse sections of the brain, obtained at 1.5-mm intervals, are arranged in a rostral (a) to caudal (e) direction to illustrate the approximate position of the 444 labelled neurones (\bullet) observed in 20 grafted animals. The arrow in d indicates the site of 15 graft insertions made stereotactically 4.4 mm rostral to the inter-aural line in the horizontal plane¹⁹. In five other animals the graft was inserted 9 mm rostral to and 2 mm above these two reference lines (arrow in a).

these cells to regenerate in the experimental conditions used, or may simply be the consequence of their proximity to the transplanted nerve; different graft locations may elucidate this question. Most of the labelled cerebral neurones were situated near the tip of the graft, which suggests that the remarkable elongation of their axons results from local sprouting triggered by injury 13-15 resulting from the grafting procedure and facilitated by growth-favouring conditions in the PNS transplant. It is also possible that growth factors released by the segment of nerve 16,17 may influence only the soma of nearby cells. The demonstration that the cerebral neurones that give rise to the re-growing axons are confined to an area within a few millimetres of the end of the graft is consistent with similar observations in the rat spinal cord and brain stem10,11. These findings suggest that it may be possible to use similar grafting methods to investigate further neuronal plasticity in other specific areas of the CNS of mature and immature animals and in conditions associated with ageing and disease.

Although the axons of neurones labelled in these experiments would, in the intact animal, reside and project exclusively within the CNS, they have successfully elongated along the implanted grafts in a manner similar to that of fibres injured in peripheral nerves. This indicates that such elongation may depend on properties shared by populations of intrinsically and extrinsically projecting nerve cells, the potential for the growth of intrinsic CNS neurones being expressed when the neuroglial environment in the brain is substituted by that in peripheral nerves. It is not known, however, whether the cells labelled in these experiments indicate a more general potential for regeneration by CNS neurones when interacting with Schwann cells and other PNS components.

These results also suggest that in conditions created by nerve grafting, the axons of some CNS neurones can grow to lengths greater than those normally reached in the intact animal. Axons from the striatal neurones, which are thought normally to project only to the nearby globus pallidus and SN¹⁸, appear to have grown along the entire length of the grafts. Furthermore, because the intracerebral tip of the 13-mm-long graft was as far as 6.5 mm from the labelled SN cells in some animals, we

calculate that the axons from nigral neurones must have attained a total length of almost 20 mm. The extent of this axonal growth can be placed in perspective by comparing it with the maximum direct distance between the SN and the cerebral cortex: 14–15 mm in 300 g rats¹⁹.

The hypothesis that the microenvironment at the tip of the

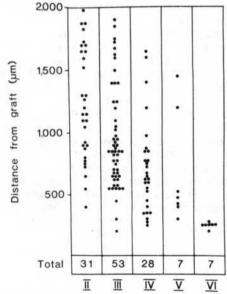


Fig. 2 Distribution of 126 HRP-labelled neurones in layers II-VI of the somatosensory cortex of grafted rats. The vertical axis indicates the distance between the labelled soma and the intracerebral tip of the graft. The cells in the more superficial layers are nearly 2 mm from the graft. The diameter of the labelled cortical neurones averaged 24.9 \pm 4.05 μm (mean \pm s.d.) (range 16.8-38.4 μm) while that of 250 unlabelled neighbouring cells was 17.9 ± 3.63 μm (range 12.0-28.8 μm). The difference in size between the labelled and unlabelled cells may result from a neuronal reaction to axon interruption at the time of HRP application to the graft.

re-growing axon has a decisive role in the success or failure of regeneration implies that non-neuronal PNS components influence neuronal mechanisms that regulate elongation²⁰ whereas the CNS milieu of adult mammals either lacks these growth-promoting factors or exerts an inhibitory influence. The critical differences between the CNS and PNS microenvironment are unknown but may include specific substances released by cells^{6,22}, influences arising from extracellular components²³, surface properties of sheath cells²⁴ and, possibly the different spatial arrangement of glia in the CNS and PNS. Further questions which require investigation are whether central axons elongate into PNS grafts by the regrowth of the damaged fibres, by collateral sprouting or by both. Will long projecting axons

such as those of the corticospinal tract, also grow along PNS grafts after they are interrupted in the brain? What connections, if any, can be established between the growing CNS axons and the target tissues to which the nerve graft is attached? Our findings suggest that the elongation of axons from these central neurones is governed by interactions between the growing nerve fibres and their surrounding tissues, and that abortive regeneration is not the result of an intrinsic lack of neuronal potential to support renewed growth.

This work was supported by grants from the MRC of Canada, the Muscular Dystrophy Association of Canada and the Multiple Sclerosis Society of Canada. M.B. held a studentship from the Faculty of Medicine of McGill University.

Received 11 November 1981, accepted 14 January 1982

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Functional synapses are established between ciliary ganglion neurones in dissociated cell culture

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The formation of specific synaptic connections is a central part of development in the nervous system. The parasympathetic ciliary ganglion of the chick is a useful population of neurones for studying synaptogenesis both because its development has been well characterized in vivo¹ and because its neurones can be maintained and examined in long-term dissociated cell culture^{2,3}. The ciliary ganglion contains two classes of neurones: choroid neurones that innervate smooth muscle in the choroid layer of the eye, and ciliary neurones that innervate striated muscle in the iris and ciliary body. Both classes of neurone are cholinergic and both receive excitatory cholinergic input from preganglionic neurones in the accessory oculomotor nucleus. Ciliary ganglion neurones do not seem to innervate each other in $vivo^{4.5}$, even though they have matching neurotransmitter and receptor types. In cell culture, the neurones acquire high levels of choline acetyltransferase activity, form cholinergic synapses on skeletal myotubes when present in the cultures, and have significant levels of acetylcholine (ACh) sensitivity2,6, as they do in vivo. We report here that cholinergic synaptic transmission does occur between the neurones in cell culture. These results indicate that ciliary ganglion neurones can innervate each other, and suggest that additional constraints exist in vivo to prevent them from doing so.

Dissociated ciliary ganglion neurones were obtained from $7\frac{1}{2}$ -day-old chick embryos and, unless otherwise indicated, were grown in 35-mm dishes with skeletal myotubes as previously described². In some cases the neurones were grown alone, using a collagen substratum coated with fibroblast material². Culture medium was as previously described for nerve-muscle cultures² except that some cultures of neurones alone received medium with 3% (v/v) embryonic chick eye extract instead of 5% (v/v) whole embryo extract². After 5-14 days the neurones were

examined using intracellular recording techniques2,6; microelectrodes were filled with 1 M potassium acetate and had tip resistances of 80-200 M Ω . Recordings were accepted only if impaled neurones had resting potentials exceeding -45 mV and fired impulses in response to intracellular stimulation.

Intracellular recordings from the neurones revealed discrete spontaneous depolarizations that ranged in amplitude over 0.5-15 mV and in half rise time over 0.6-1.2 ms, and appeared to decay exponentially (Fig. 1). The frequencies of such depolarizations among neurones varied from 2 to 300 per min and persisted throughout recordings that lasted up to 45 min. At high frequencies the depolarizations often summed and occasionally triggered action potentials in the neurones (Fig. 1). Over 90% of the neurones grown with myotubes displayed such depolarizations whereas only 42% of the neurones grown alone did so (Table 1).

Pharmacological experiments indicated that the spontaneous depolarizations were cholinergic in origin. Perfusion of the cultures with 25 μM(+)tubocurarine (TC) completely abolished the depolarizations in all neurones within 5 min; partial recovery of the spontaneous activity was obtained 4 min after washout of the drug was initiated (Fig. 2). After 30 min of washout, the occurrence of depolarizing potentials among randomly selected, treated neurones was indistinguishable from that obtained in control cultures (Table 1).

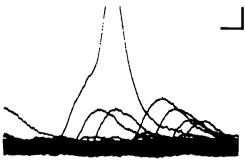


Fig. 1 Spontaneous depolarizations recorded in a ciliary ganglion neurone grown with myotubes. A number of oscilloscope traces have been superimposed showing about 15 depolarizations. In one case a depolarization has triggered an action potential. Resting potential: -61 mV. Calibration bars: vertical, 5 mV; horizontal, 2 ms.



Fig. 2 Reversible blockade of the spontaneous depolarizations by (+)tubocurarine. a, Spontaneous depolarizations in a ciliary ganglion neurone grown with myotubes; b, blockade of the depolarizations in the same neurone 4 min after the initiation of perfusion with 25 μM TC; c, recovery of the depolarizations in the same neurone 4 min after washout of the TC was initiated by perfusion with normal medium. Resting potential: -60 mV. Calibration bars: vertical, 20 mV; horizontal, 2 s.

The spontaneous depolarizations were also blocked by the α -neurotoxin Bgt 3.1 which has previously been shown to block the ACh sensitivity of ciliary ganglion neurones in cell culture^{6,7}. Neurones incubated with 20 nM Bgt 3.1 for 1 h at 37 °C were never seen to display the spontaneous depolarizations (Table 1). Recovery of the responses was observed after removal of the Bgt 3.1 (Table 1). During TC or Bgt 3.1 treatment, the neurones remained able to fire action potentials when stimulated intracellularly, and had resting potentials not significantly different from those recorded in normal medium (P > 0.25, Student's t-test).

 α -Bungarotoxin (Bgt 2.2), which inhibits ACh receptors on muscle cells but not ACh receptors on ciliary ganglion neurones⁶, did not block the spontaneous depolarizations observed in the neurones. After a 1-h incubation in 10 nM Bgt 2.2, most of the neurones tested still displayed the spontaneous potentials (Table 1). The Bgt 2.2 treatment was effective, however, at blocking muscle ACh receptors, because the normally twitching myotubes in the nerve-muscle cultures were quiescent after the incubation with Bgt 2.2. Intracellular recording demonstrated that the synaptic potentials normally observed in the myotubes were absent. These results indicate

Table 1 Incidence and pharmacological blockade of spontaneous depolarizations in ciliary ganglion neurones

Culture type	Drug treatment	% Neurones with spontaneous depolarizations
Nerve-muscle	None	92 (60, 12)
Nerve	None	42 (24, 8)
Nerve-muscle	TC	0 (13, 6)
	TC removed	93 (14, 6)
Nerve-muscle	Bgt 3.1	0 (13, 3)
	Bgt 3.1 removed	83 (6, 2)
Nerve-muscle	Bgt 2.2	82 (11, 2)

Ciliary ganglion neurones were grown with skeletal myotubes (nervemuscle) or grown alone (nerve), and incubated with the indicated drug for 1 h at 37 °C, and then examined in the same conditions for spontaneous depolarizations as described in the text for a period of at least 3 min per neurone. The mean resting potential was -56 ± 1 mV (\pm s.e., n = 80). The proportion of neurones with spontaneous depolarizations is presented as a per cent of the neurones tested; the number of neurones tested and the number of cultures sampled are shown in parentheses. Drug concentrations were TC, 25 µM; Bgt 3.1, 20 nM; and Bgt 2.2, 10 nM. For one of the TC cultures, 25 µM hexamethonium was included and the TC concentration was 100 µM. Recovery from TC treatment was followed in the same cultures used to test the effects of TC: at the end of the TC test period the cultures were rinsed and incubated for 0.5 h at 37 °C and then re-examined (TC removed). As for recovery from Bgt 3.1, cultures previously tested in Bgt 3.1 were rinsed and incubated for 2 h at 37 °C and then re-examined (Bgt 3.1 removed). In all cases the cultures were also tested before drug treatment to verify that the neurones displayed the expected incidence (~90%) of spontaneous depolarizations.

that the spontaneous depolarizations observed in the neurones are not in some way dependent on neurogenic muscle contraction, a conclusion also implied by the presence of the depolarizations in neurones grown alone.

The pharmacological experiments indicate that the spontaneous depolarizations recorded in the neurones represent cholinergic excitatory postsynaptic potentials. They can be reversibly blocked by TC and Bgt 3.1, and are not blocked by Bgt 2.2. A similar pharmacology has been described for ACh receptors on the neurones⁶. The implication is that ciliary ganglion neurones establish synapses on each other in cell culture and that neurotransmitter release can occur spontaneously or be evoked by spontaneous action potentials in the presynaptic neurone. Synapses between ganglionic neurones have also been described for rat sympathetic neurones in cell culture when the cells are induced to become cholinergic^{8,9}.

Morphological evidence for chemical synapses between ciliary ganglion neurones in culture has been reported previously, but stimulation of the neurones failed to evoke synaptic transmission¹⁰. We routinely test neurones for electrical excitability by using intracellular stimulation to trigger action potentials. We have occasionally seen depolarizations produced in a neurone following action potentials elicited in the same neurone. The evoked depolarizations were phase-locked to the action potentials with a fixed time delay and, in the case of one neurone, were adequate to elicit a second action potential (Fig. 3). Although we have not demonstrated that the evoked depolarizations can be blocked by cholinergic antagonists, they do have the range of amplitude and half rise time described above for the synaptic potentials. Studies with tetrodotoxin (TTX) support the possibility that evoked transmission does occur between the neurones. Perfusion of nerve-muscle cultures with 10 μM TTX for 2 min completely abolished the spontaneous synaptic potentials (eight neurones, three cultures); rinsing the cultures with medium lacking TTX for 4 min resulted in the reappearance of synaptic potentials in four out of five neurones tested (three cultures). These results are consistent with the synaptic potentials being elicited by spontaneous TTX-sensitive action potentials which we frequently observed while recording from the neurones (unpublished observations). To determine whether evoked transmission can be reliably obtained at synapses capable of spontaneous transmission, however, it will be necessary to identify transmitting neurone pairs and to test them with intracellular stimulation.

The difference in the proportion of neurones showing synaptic potentials in nerve compared with nerve-muscle cultures (Table 1) could reflect differences in the frequency of action

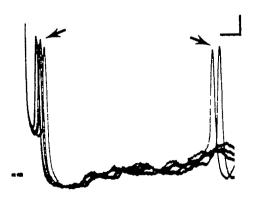


Fig. 3 Recordings from a neurone grown with myotubes showing evoked depolarizations occurring with a fixed time delay after an action potential. Five superimposed oscilloscope traces are shown. In each case a brief (0.1 ms) intracellular current pulse was used to trigger an action potential (arrow, left) in the impaled neurone. After a delay of ~10 ms, a series of depolarizations was observed. In two of the five trials the depolarizations triggered a second action potential (arrow, right). The nearly vertical line at the far left marks the stimulus artefact. Resting potential: -58 mV. Calibration bars: vertical, 10 mV; horizontal, 5 ms.

potentials in presynaptic neurones or differences in the number or efficacy of synapses in the two kinds of cultures. The presence of myotubes as a synaptic target for the neurones clearly does not prevent the neurones from also forming synapses on each other.

Although it remains possible that ciliary ganglion neurones transiently innervate each other at early stages in vivo, it seems clear that they normally do not do so in the adult chick or pigeon^{4,5}. Recently it has been shown that parasympathetic neurones of the frog cardiac ganglion which also do not normally innervate each other, can do so in vivo when deprived of their normal preganglionic input; reinnervation of the neurones by the preganglionic fibres leads to suppression of the synapses between ganglionic neurones¹¹. The formation of synapses between ciliary ganglion neurones in culture may also reflect the absence of their normal preganglionic fibres.

This work was supported by grants from the NIH (NS 12601 and MH 16109) and from the Muscular Dystrophy Association. We thank Dr Martin Smith for providing some of the data obtained with Bgt 3.1, Marie Ryder for providing technical assistance, and Dr Nicholas Spitzer for comments on the manuscript.

Received 24 August 1981, accepted 11 January 1982

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Growth factors adherent to cell substrate are mitogenically active in situ

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We report here that platelet-derived growth factor (PDGF)1-4 and pituitary-derived fibroblast growth factor (FGF)5 bind rapidly and tenaciously to tissue culture plates either coated with collagen (a major component of the extracellular matrix) or uncoated. Surface-bound PDGF resists extraction with nonionic detergents, organic solvents and chaotropic solvents: moreover it is biologically active in situ. Our finding that immobilized PDGF is nonetheless mitogenic lends support to other observations which indicate that PDGF functions outside the cell via production of a stable intracellular second signal⁶. In a broader context, the observations suggest that one role of the extracellular matrix in vivo is to sequester biologically active molecules like PDGF to provide a localized and persistent

PDGF was purified to homogeneity and radioiodinated as described in Table 1 legend. Binding studies with 125I-PDGF (see Table 1 legend) showed that the radiolabelled material binds to specific receptors on the surface of confluent BALB/c-3T3 cell monolayers. We and others have found that most of

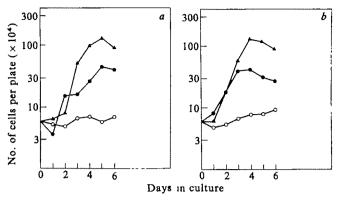


Fig. 1 PDGF-treated plates support continued proliferation of BALB/c-3T3 cells in medium containing platelet-poor plasma. Tissue culture plates (35 mm), either coated with collagen or uncoated, were exposed to 500 units ml⁻¹ of heat-treated PDGF³ at 37 °C for 4 h. Control plates were not treated with PDGF. BALB/c-3T3 cells (6×10^4) were seeded on to each plate in medium containing 5% platelet-poor plasma and cell numbers were determined daily; cells were fed every other day. For comparison, the growth of cells on untreated plates in medium containing 5% calf serum is shown. a, Collagen-coated plates: •, 5% plasma, PDGF-treated plates; O, 5% plasma, untreated plates; A, 5% serum, untreated plates. b, Uncoated plates: symbols as in a.

the 125I-PDGF bound to confluent monolayer cell cultures is cell associated. In the absence of a cell monolayer, however, 12 PDGF binds to the surface of empty tissue culture plates in a non-saturable manner. In the experiments summarized in Table 1, 125I-PDGF (20 ng ml-1) was placed over the surface of empty microtitre tissue culture wells and incubated at 37 °C for 1 h. In these conditions about 1 ng of PDGF becomes associated with the surface of the culture dish, and this surface-bound PDGF resists extraction with various strong solvents. We found that boiling SDS is partially effective in extracting the bound PDGF, and pronase solubilizes ~70% of the radioactivity. From the molecular weight of PDGF (33,000-35,000)3.4 it can be calculated that the empty tissue culture wells bind between 6×10^{10} and 9×10¹⁰ molecules of PDGF per cm². Although the absolute amount of PDGF bound is small, a 3T3 cell (spread surface area 2×10^{-5} cm²) plated on to a culture dish pretreated with PDGF could be presented with between 3×10^5 and 4.5×10^5 molecules of PDGF from below. This is well in excess of the number of molecules needed to saturate cellular PDGF receptors (see Table 1 legend).

When quiescent 3T3 cells were plated out in culture dishes that had been pretreated with PDGF, DNA replication was induced in the absence of any PDGF added to the medium (Table 2), thus PDGF bound to the surface of tissue culture dishes is mitogenically active. F6F, which is a functional analogue of PDGF for stimulating the growth of BALB/c-3T3 cells⁸, also retained its mitogenic activity when bound to tissue culture dishes (Table 2). The concentrations of pure PDGF or partially pure FGF which delivered a mitogenic quantity of growth factor to the surface of empty tissue culture plates (Table 2) were of the same order of magnitude as those used to stimulate cell growth in conventional conditions^{3,5}. The mitogenic activity of PDGF fixed to the surface of tissue culture plates resisted extraction with 6 M urea, methanol, ethanol and glycine-EDTA (pH 10) as well as Triton X-100 (Table 3), but was totally abolished by reduction with 2-mercaptoethanol or digestion with proteases, as is the activity of soluble PDGF3. In agreement with the data of Table 1, boiling 1% SDS partially removed or inactivated the surface-bound PDGF.

We demonstrated that surface-bound PDGF is biologically active in situ in the following way. One-half of an empty tissue culture plate was exposed to PDGF by tilting the plate during treatment. The plate was washed with phosphate-buffered saline (PBS) and then 3T3 cells were seeded over the entire plate at a density of 7×10^4 cm⁻² in medium containing 5% plasma

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and ³H-TdR. The culture plate was incubated for 42 h, fixed and processed for autoradiography. The amount of labelling on the treated side of the plate was 42%, that on the untreated side 7%. Hence, the mitogenic activity of PDGF-treated plates is predominantly a local effect.

Surface-bound PDGF supports repeated cycles of cell growth and division as well as localized induction of DNA synthesis. We compared proliferation of BALB/c-3T3 cells on four substrates (plastic, collagen, plastic pre-treated with PDGF, and collagen pre-treated with PDGF) in medium containing 5% plasma (Fig. 1). Cells maintained on plastic or collagen proliferated only

Table 1 Binding of ¹²⁵I-PDGF to the surface of tissue culture dishes

PDGF-treated plates extracted with:	c.p.m. ¹²⁵ I remaining on surface of dish
PBS	10,806
Triton X-100 (0.5%)	11,389
Methanol (95%)	12,896
Ethanol (95%)	12,362
Urea (6 M)	15,495
SDS (1%) (100 °C)	7,963
Pronase (2 mg ml ⁻¹)	3,760

PDGF was purified to homogeneity by a modification of our previous method³, then radioiodinated to a specific activity of 10,000 d.p.m. ng⁻¹ by a modification of the Hunter and Greenwood procedure¹⁸. The 125 I-PDGF retained its mitogenic activity. Studies of binding specificity to BALB/c-3T3 cells (performed at 4 °C) indicated that confluent monolayer cultures of BALB/c-3T3 possesses $\sim 8\times 10^4$ PDGF receptors per cell. Nanogramme per ml quantities of unlabelled PDGF completely displaced the 125 I-PDGF from these surface receptors whereas microgramme per ml quantities of epidermal growth factor or insulin had no effect. To measure the binding of 125 I-PDGF to the cell substratum, empty microtitre tissue culture wells (0.3 cm² surface area) were incubated with 40,000 c.p.m. of 125 I-PDGF in 200 µl Dulbecco's modified Eagle's medium (DMEM) with 1 mg ml $^{-1}$ of unlabelled bovine serum albumin as carrier. After 1 h.at 37 °C the 125 I-PDGF solution was aspirated. The microtitre plates were then extracted at 37 °C for 1 h with the indicated solvents (except for SDS which was a 1 min extraction at 100 °C). Following extraction the solvents were aspirated. The microtitre plates were again washed extensively with PBS and dried. The bottom of each microtitre plate was punched out and placed in a γ counter for determination of bound 125 I-PDGF.

Table 2 Mitogenic activity of surface-bound PDGF and FGF

	y plates with PDGF		y plates I with FGF
[PDGF] (ng ml ⁻¹)	% Labelled nuclei	[FGF] (ng ml ⁻¹)	% Labelled nuclei
40 20 10	94.9 95.2 83.2	1,000 300 100	91 65 58
2.5 0	65.3 24.5	30 0	8 2

The mitogenic activity of surface-bound PDGF and FGF was detected by induction of DNA synthesis in quiescent 3T3 cells. Empty microtitre tissue culture wells were precoated with collagen (collagen dispersion TD-150: Ethicon, Massachusetts) to facilitate rapid cell attachment. The collagen-coated plates were then pretreated with the indicated concentrations of electrophoretically pure PDGF (prepared by a modification of a previous protocol3) or partially purified pituitary FGF (KOR Biochemicals, Massachusetts) at 37 °C for 4 h. The plates were rinsed twice with PBS and further extracted at 37 °C for 1 h with 0 5% Triton X-100 as described in Table 1 legend. Quiescent density-arrested BALB/c-3T3 cells were collected by trypsin digestion and immediately replated at a density slightly greater than their confluent density (\sim 7× 10⁴ cells cm⁻²) into the treated microtitre culture wells. The cultures were incubated at 37 °C for 36 h in medium containing ³H-thymidine (5 μC₁ ml⁻¹) and 5% platelet-poor plasma (which lacks PDGF¹) cultures were then fixed and processed for autoradiography. Per cent labelled cells was determined for each well.

Table 3 Mitogenic activity of surface-bound PDGF after extraction with various solvents and enzymes

PDGF-treated plates extracted with:	% Labelled nuclei ±s.d.
Triton X-100 (0.5%)	$66 \pm 20 \ (n=8)$
Glycine (10 mM; pH 10.0)	88±5 (3)
Methanol	$92 \pm 5 (2)$
Ethanol	$84 \pm 4 (2)$
Urea (6 M)	$68 \pm 17 (3)$
RNase $(30 \mu \text{g ml}^{-1})$	$77 \pm 5 (2)$
DNase $(10 \mu \text{g ml}^{-1})$	$74 \pm 5(2)$
Acetic acid (1 M) (pH 2.3)	$46 \pm 12 (3)$
SDS (1%; 100°C)	$40 \pm 32 (3)$
Urea (6 M) +2-mercaptoethanol (10%)	$10\pm 8(2)$
Pronase (2 mg ml ⁻¹)	$0 \pm 5(3)$
Trypsin (1,000 U ml ⁻¹)	$19 \pm 17(2)$

The surface of empty tissue culture plates was exposed to partially purified PDGF³ in DMEM at 37 °C for 3-4 h. The PDGF concentrations used were in the range 50-500 units ml⁻¹ where 1 unit of activity = ~0.2 ng PDGF³. Control plates were treated with DMEM alone. After 3-4 h the solutions were aspirated. The empty wells were then washed and extracted with solvents as described in Table 1 legend. Surface-bound, biologically active PDGF was detected by induction of DNA synthesis in quiescent 3T3 cells as described in Table 2 legend. The nuclear labelling index of cells cultured on the treated plates is shown after subtracting the background of labelled nuclei on untreated plates; the background labelling ranged from 0 to 25% in individual experiments.

poorly in 5% plasma but grew rapidly for 3–5 days on substrate pre-treated with PDGF. Cells cultured with 5% plasma on PDGF-treated plates grew to slightly lower final densities than cells cultured conventionally in serum-containing medium; however, the cells cultured conventionally received three separate PDGF treatments (through the change of serum-containing medium on alternate days) whereas the cells cultured on PDGF-treated plates were subject to only one PDGF treatment. We have obtained similar results by treating collagen-coated plates with pituitary FGF.

We conclude that PDGF and FGF adhere tenaciously to the surface of tissue culture plates; from this position, they can stimulate proliferation of cells maintained in platelet-poor plasma. These results may have a bearing on tissue culture analyses of extracellular matrix materials, and their role in controlling cell growth and function⁹⁻¹¹. The findings may also be relevant to the well characterized 'anchorage requirement' for fibroblast growth in vitro 12-14. It remains to be determined whether this has physiological relevance in vivo.

The induction of cell division by surface-bound PDGF is a highly efficient process as quantified by dose-response analysis, amount of PDGF consumed and duration of effect. If efficiency can be taken as a sign of biological relevance, these data suggest that fibroblast-like cells are intended to respond to PDGF presented in this manner. A functional interaction between PDGF and the cellular substratum in vivo can easily be rationalized. PDGF is a connective tissue mitogen which is also chemotactic for smooth muscle cells¹⁵; it is believed to have a role in wound healing and maintainence of the vascular system and may be involved in the genesis of atherosclerosis^{2,16,17}. If PDGF adheres to collagen surrounding the smooth muscle cells at the site of a wound, the mitogenic effect might be considerably prolonged. A gradient of PDGF concentration so produced (high near the wound, lower further away) might provide a directional cue for migrating cells. More generally, the adhesion of biologically active polypeptides to the extracellular matrix might serve to guide cell migration during embryogenesis or even provide a 'memory' of past developmental signals.

This research was supported by the NCI. J.C.S. is a NATO postdoctoral fellow. C.D.S. is supported by a Faculty Research Award from the American Cancer Society. We thank Lynne Dillon for typing the manuscript.

Received 6 July 1981, accepted 14 January 1982

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Selective effect of feline leukaemia virus on early erythroid precursors

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Feline leukaemia virus (FeLV), a horizontally transmitted retrovirus, is the aetiological agent of feline lymphosarcoma and leukaemia¹⁻³. In addition, the establishment of persistent FeLV viraemia in cats has been associated with the induction of a wide range of non-neoplastic conditions including immunosuppression, reproductive disorders and anaemias 4.5. FeLV isolates from cats may be divided into three subgroups, A, B and C, based on their interference patterns^{6,7}. In natural isolates, FeLV-C is always found in association with subgroups A or AB but the subgroup C component may be separated as a non-defective virus capable of establishing a persistent viraemia in neonatal cats. Pure red cell hypoplasia (PRCH) is one of three primary forms of FeLV-associated anaemia recognized in cats^{8,9} and we report here that three new biologically cloned FeLV-C isolates will reproduce this disease. We have demonstrated that infection of cats with FeLV-C/Sarma results in rapid depletion of early erythroid precursors (burst-forming units-erythroid; BFU-E) while the proportion of the myeloid precursor (granulocyte-macrophage colony-forming cells; GM-CFC) in the bone marrow remains normal.

In a survey of FeLV subgroups in naturally infected, viraemic cats, FeLV-C was not observed in 98 clinically normal animals but was isolated from 9 of the 32 (28%) anaemic cats examined. Subgroup C viruses from three of these anaemic cats were biologically cloned and used to infect neonatal kittens as described in Table 1 legend. Each isolate produced a PRCH that was clinically evident 3-6 weeks after infection. The pathology of the disease produced by these isolates resembled that previously described for FeLV-C/Sarma¹⁰

To determine the effect of subgroup C viruses on haematopoietic precursor cells, 3.5×10⁵ focus-inducing units (FIU) of FeLV-C/Sarma were inoculated intraperitoneally, within 24 h of birth, into specific pathogen-free randomly bred cats. A control group of uninfected cats was established from the same colony and members of both groups were bled weekly to determine their blood constituents and virus status¹¹. We detected viraemia in all the FeLV-infected cats except cat 7.

The control cats showed normal feline blood development¹² in that the haematocrit rose from a low value at 4 weeks until the adult level was attained at ~12 weeks (Fig. 1). In the infected cats, however, the rise in the haematocrit was not sustained but began to fall from 7 weeks onwards. Individual FeLV-Cinfected cats displayed episodes of splenic erythroid regeneration with the release of normoblasts into the circulation. These events were associated with a temporary slight rise in the individual's haematocrit before it fell to a new low level. Cat 7, which was not detectably viraemic, displayed normal blood development throughout the experiment.

Starting 3 weeks after infection and at intervals thereafter, the femoral bone marrow of the cats was assayed for the early erythroid precursor (BFU-E)¹³ and the neutrophil macrophage precursor (GM-CFC; refs 14, 15) (Table 2). The numbers of BFU-E and GM-CFC in all the control cats were within the range previously established for cats 3–12 months old (data not shown). Examination of the FeLV-C-infected cats, however, indicated that a dramatic reduction in the numbers of BFU-E cells had occurred as early as 3 weeks after infection while the incidence of GM-CFC was normal (Table 2). A reduction in BFU-E numbers in FeLV-C-infected cats has also been observed by others (J. Adamson, personal communication).

Both control and FeLV-C-infected cats exhibited splenic extramedullary haematopoiesis (EMH) which is a normal feature of feline haematopoiesis in cats of this age range. This process may have accounted for the normal haematological status of cat 7, which was not detectably viraemic but which showed extensive reduction of BFU-E in the bone marrow. The pattern of reduced BFU-E and normal GM-CFC levels was seen in the infected cats throughout the experiment, except

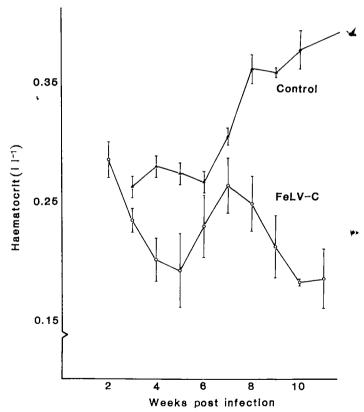


Fig. 1 Mean haematocrits (±s e) of control uninfected and FeLV-Sarma-infected kittens. A control group of 6 newborn kittens and 10 FeLV-C/Sarma-infected newborn kittens were established from the same colony. A separate group of three control and eight of the FeLV-C/Sarma-infected kittens were used in the colony assays described in Table 2 (cats 1-11). The kittens were bled weekly by jugular venepuncture and a 1-ml blood sample was used to establish the haematological values and the presence or absence of plasma viraemia as previously described¹¹ A comparison of the haematocrits of the FeVL-C-infected and control group by Wilcoxon's two-sample rank test indicated significant differences (0 01 level of T) at weeks 5, 8 and 9. From week 10 onwards only two kittens remained in the FeLV-C-infected group, precluding a statistical comparison.

Table 1 Development of the haematocrit and total leukocyte count in FeLV-C-infected cats

		Haematocrit ($l l^{-1} \pm s.e.$)				
	Control	FeLV-C/ FS246	FeLV-C/ FA27	FeLV-C/ FZ215	FeLV-C/ Sarma	Control
Age (weeks)						
3	0.262 ± 0.009	0.250 ± 0.007	0.254 ± 0.008	0.197 ± 0.016	14.0 ± 1.6	14.8 ± 3.1
4	0.279 ± 0.009	0.234 ± 0.003	0.196 ± 0.005	0.209 ± 0.009	14.9 ± 2.1	14.7 ± 2.6
5	0.274 ± 0.009	ND	0.205 ± 0.013	0.149 ± 0.017	17.6 ± 5.3	15.7 ± 1.9
6	0.276 ± 0.005	0.184 ± 0.006	0.213 ± 0.015	0.127 ± 0.012	15.3 ± 1.7	13.2 ± 2.5
7	0.304 ± 0.007	0.202 ± 0.010	0.256 ± 0.090	0.129 ± 0.015	12.7 ± 2.7	12.4 ± 1.8
8	0.394 ± 0.026	0.205 ± 0.015	0.258 ± 0.011	0.125 ± 0.023	21.0 ± 4.5	18.3 ± 2.9
9	0.358 ± 0.004	0.213 ± 0.026	ND	0.123 ± 0.026	16.8 ± 3.2	14.5 ± 1.9
10	0.376 ± 0.017	0.188 ± 0.028	ND	0.092 ± 0.024	17.4 ± 1.5	19.1 ± 2.2
11	ND	0.185 ± 0.022	0.258 ± 0.014	***	21.2 ± 1.9	24.7 ± 3.0
12	0.394 ± 0.009	0.187 ± 0.028	0.248 ± 0.024	-	_	_
13	ND	0.185 ± 0.023	0.231 ± 0.030		-	
14	0.358 ± 0.011	0.172 ± 0.017	0.182 ± 0.040		_	+
15	ND	0.159 ± 0.019	$0.180* \pm 0.044$	_	-	_
16	0.371 ± 0.008	0.136 ± 0.017	0.197 ± 0.050	-	_	

Three new FeLV-C isolates, FZ215, FS246 and FA27, were biologically cloned, checked for subgroup-C specificity by interference assay and the titre determined as described previously¹¹. The pathogenicity of these isolates was determined by intrapernoneal inoculation to groups of four 1-day-old kittens with either 3.4×10^5 FIU of FZ215, 1.0×10^4 FIU of FS246 or an undetermined titre of FA27 (ref. 23). A group of six age-matched controls remained uninfected. The haematocrit and plasma viraemia were determined each week for 3 weeks. In a separate experiment (described in Fig. 1 legend) day-old kittens were either inoculated with FeLV-C/Sarma or uninfected (controls) and the weekly total leukocyte counts measured. The haematocrit and bone marrow colony assays for this experiment are given in Fig. 1 and Table 2, respectively. ND, not determined. — Experiment completed.

for cat 10 which, when killed at 11 weeks, had reduced GM-CFC. There may have been a limited effect of FeLV-C on GM-CFC but a simple interpretation of this effect was complicated by the occurrence of myelofibrosis and osteosclerosis. Although there was a marked reduction in total bone marrow cellularity in FeLV-C/Sarma-infected cats, both these cats and the controls exhibited a progressive rise in leukocyte numbers (Table 1), typical of kittens of this age¹². There is therefore a clear selective effect of FeLV-C in ablating the precursors of erythroid cells compared with those of myeloid cells and this effect is reflected in the circulating numbers of end cells of each of these compartments.

Myelofibrosis and medullary osteosclerosis have been described as accompanying features of experimentally produced PRCH¹⁶. These features were also seen in the present experiments which used cloned FeLV-C isolates but the extent and time of development of the conditions displayed considerable individual variation. In the experiment with FeLV-C/Sarma, early histopathological signs of myelofibrosis and osteosclerosis were detectable in the infected cats after 5 weeks but were absent from those examined at 7 weeks. At 11 weeks, cat 10 showed a marked development of both medullary osteosclerosis and myelofibrosis not evident in cat 11. It is apparent, therefore, that the anaemia associated with FeLV-C infection is not due to myelophthisis, as the former can occur in the absence of the myelofibrosis-osteosclerosis complex. It is also unlikely that the osteosclerosis and myelofibrosis are secondary consequences of the anaemia since osteosclerosis is not a feature of feline anaemias induced by other agents.

It is possible that some PRCH cases are associated with FeLV-A or FeLV-AB infections. However, the pronounced association of FeLV-C with anaemia and the ability of all four cloned FeLV-C isolates to induce PRCH suggest that this disease is predominantly associated with this subgroup. Furthermore, none of the FeLV-A or FeLV-B isolates tested in pathogenesis experiments have produced PRCH although other forms of anaemia have been reported. 11,17.

The mechanism of FeLV-C-induced depletion of BFU-E is unknown. It does not appear to be associated with a hyporesponsiveness of BFU-E in infected marrow to erythropoietin as levels of erythropoietin five times above the plateau level do not increase numbers of BFU-E observed (Table 2). The

Table 2 Colony forming units of erythroid and myeloid precursors in normal and FeLV-C-infected bone marrow

Age (weeks)	Cat	Status	BFU-E	GM-CFC
3	1	FeLV-C/Sarma	2.0 ± 1.6	62.0±9.4
	2	FeLV-C/Sarma	1.0 ± 1.0	74.0 ± 13.6
	3	Control	43.0 ± 8.2	72.0 ± 8.4
5	4	FeLV-C/Sarma	1.6 ± 1.6	57.0 ± 12.2
	5	FeLV-C/Sarma	74 ± 18	47.0 ± 11.4
6	6	Control	30.6 ± 1.0	61.6 ± 5.8
7	7	FeLV-C/Sarma	6.0 ± 1.6	48.0 ± 11.0
	8	FeLV-C/Sarma	22.0 ± 4.4	39.4 ± 56
8	9	Control	72.0 ± 1.6	46.0 ± 6.2
11	10	FeLV-C/Sarma	12.0 ± 6.2	14.0 ± 2.4
	11	FeLV-C/Sarma	2.6 ± 2.5	31.0 ± 5.0
36	12	Control (0 unit Epo)	0	
		Control (0.05 unit Epo)	94±18	35.4 ± 3.7
		Control (0.10 unit Epo)	28.6 ± 1.4	374±68
		Control (0.2 unit Epo)	28.0 ± 9.8	40.6 ± 5.8
		Control (0.5 unit Epo)	24.0 ± 5.4	36.0 ± 3.0
36	13	FeLV-AC (0 1 unit Epo)	1.4 ± 1.4	50.6 ± 4.8
		FeLV-AC (0.5 unit Epo)	0.6 ± 0.6	50.6 ± 12.4

Colony forming units of erythroid (BFU-E) and myeloid (GM-CFC) precursors per 105 bone marrow cells from normal FeLV-C-infected cats. The haematocrits and total leukocyte counts of cats 1-11 are given in Fig. 1 and Table 1, respectively. Cats 12 and 13 were used in a separate experiment to determine the effect of the erythropoietin (Epo) concentration on BFU-E formation, a different batch of anaemic mouse serum was used in these experiments. The technique used to grow crythroid bursts derived from BFU-E of cat bone marrow was modified from that described for other species²⁴. Femoral bone marrow cells suspended in alpha medium (Gibco) plus 2% fetal calf serum (FCS) were cultured in alpha medium containing nucleosides supplemented with 20% FCS (Flow), 10^{-7} M sodium selenite, 3.4×10^{-6} M human transferrin saturated with iron, 10^{-4} M thioglycerol and 1% bovine serum albumin (BSA) in 0.8% 4000 CPS methylcellulose (Dow Chemicals) The source of erythropoletin was anaemic mouse serum (AMS)²⁵ previously titrated against the International Erythropoletin Standard previously titrated against the International Erythropoietin Standard B The optimum dose for the burst assay in cats 1-11 was 0 4 U Epo per ml of culture. Four aliquots (0 25 ml) each containing $2.5-5.0\times10^4$ cells were plated in wells and incubated at 37 °C in a water-saturated atmosphere of air with 5% CO₂ for 9 days, previously shown to be optimum for the full growth of bursts A more detailed description of the technique will be reported elsewhere. Colonies of granulocytes and macrophages, derived from their progenitors, GM-CFC, were scored in the same cultures used to determine the numbers of bursts. Previous experiments showed that normal mouse serum or AMS provide an adequate stimulus for the growth of GM-CFC In these experiments, the amount of AMS used in the erythroid cultures also provided maximum stimulation for the assay of GM-CFC.

^{*} Cat removed with a haematocrit of 0.092.

decrease in BFU-E may be due to immune elimination of infected cells, to an effect on accessory cells required for erythropoiesis, or a direct block of differentiation of early erythroid precursors. Certain of the adult cases of pure red cell aplasia in man have been postulated to be an autoimmune condition mediated by antibody directed against either erythropoietin or erythroid precursors ^{18,19}. Immune elimination of infected precursors in the cat, following viral antigen expression on the surface of infected cells, seems unlikely. This follows from an examination of bone marrow smears for the presence of FeLV group-specific antigen, p30, by the indirect immunoperoxidase technique, which indicated that widespread infection of myeloid cells occurred but the incidence of GM-CFC (Table 2) and the numbers of circulating leukocytes were normal (Table 1). Furthermore, elimination of infected cells is associated with the production of neutralizing antibody and with the consequent abrogation of the infection, while the establishment of clinical anaemia is associated with a persistent infection and the absence of neutralizing antibody. Lymphoid cell infiltrations have been observed in the marrow of humans having pure red cell aplasia 19 and a lymphoid-dependent suppression of haematopoiesis has been suggested for some cases of aplastic anaemia²⁰. In the FeLV-C-infected cats, however, we did not detect any extensive lymphoid infiltration of the marrow. An effect of FeLV-C on accessory cells cannot be excluded. These cells could either represent cells forming the local microenvironment of the bone marrow or T cells which may promote erythropoiesis²¹. In our experiments a gross effect on the T-cell compartment was noted in cat 11 which had an extensive thymic atrophy, while the other FeLV-C infected cats showed no thymic depletion. In other FeLV-C experiments^{8,10} thymic atrophy has been a major finding, but FeLV of other subgroups induce this change without producing anaemia. However, a more subtle modulation of T-cell subsets may have occurred in the FeLV-C-infected cats which affected their capacity to promote or inhibit erythropoiesis.

Any hypothesis explaining the findings presented here must account for the remarkable selective effect of FeLV-C in producing a red cell hypoplasia but with normal levels of GM-CFC and circulating leukocytes. An effect of FeLV-C on the accessory cells required for erythropoiesis could be consistent with these observations but may also be explained by a mechanism that involves a direct effect of the virus on early erythroid precursor cells. The latter hypothesis is consistent with the subgroup specificity of the disease as the envelope glycoprotein, gp70, is involved both in defining the subgroup of the virus and acting as a binding protein for target cells. Unlike leukaemia and lymphosarcoma, which have latent periods of months or years, erythroid hypoplasia develops rapidly after productive infection. In FeLV-induced immunosuppression develops rapidly after infection, it has been shown that an isolated viral protein can produce many of the effects associated with this syndrome, including the suppression of lymphocyte mitogenesis²². Thus it is important to determine whether the induction of this disease is dependent on integration of the FeLV-C genome into target cells or whether the binding of viral proteins may suppress the activity of erythroid precursor or accessory cells.

We thank Margaret Booth and Mathew Golder for technical assistance. This work was supported by the CRC and the Leukaemia Research Fund.

Received 23 October 1981, accepted 26 January 1982

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Two genetic loci control the murine immune response to A-gliadin, a wheat protein that activates coeliac sprue

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Coeliac sprue is a disease in humans that is characterized by small intestinal mucosal injury and malabsorption. Ingestion of dietary gluten by susceptible individuals results in damage to the small intestine. The gliadin fractions of wheat gluten and similar proteins in rye and barley are known to activate the disease process¹. The major histocompatibility complex (MHC) alleles HLA-B8, Dw3 and DRw3 occur at a high frequency in coellac sprue²⁻⁵, suggesting that immunoregulatory mechanisms may be important in the pathogenesis of the disease. We recently reported that murine antibody responses to A-gliadin, a well characterized protein known to activate coeliac sprue1.6 are regulated by genes that map to H-2, the murine MHC10. However, it appeared that an additional genetic locus also might be important in regulating the immune response to gliadin proteins. We show here that two separate genetic regions determine the murine antibody response to A-gliadin: H-2 on chromosome 17 and the immunoglobulin heavy chain (C_H) allotype locus (Igh) on chromosome 12. Possible ways in which these two loci could determine the response against A-gliadin are discussed.

Our studies took advantage of C_H and H-2 congeneic strains of mice to assess the IgG humoral antibody response to Agliadin. Mice bearing the $H-2^d$ haplotype are high responders to A-gliadin whereas those bearing the $H-2^k$, $H-2^j$, $H-2^b$ and H-24 haplotypes are low responders10. Groups of age- and sex-matched mice were injected intraperitoneally (i.p.) with 25 µg A-gliadin in complete Freund's adjuvant (CFA), and bled 18, 29 and 42 days after immunization. IgG anti-A-gliadin antibody levels were measured by radioimmunoassay10

BALB/c $(H-2^d, Igh^a)$ and C.B20 $(H-2^d, Igh^b)$ congeneic mice have different Igh haplotypes but share the same H-2 and background genes. As shown in Fig. 1, IgG anti-A-gliadin responses in C.B20 mice were significantly lower than the BALB/c controls. Similarly reduced anti-A-gliadin responses were seen in the BALB/c Igh congeneic C.AL20 strain (H-2^d Igh^d). The C.B20.B and C.B20 congeneic strains differ only at ~ H-2 whereas the C.B20.B and BALB/c congeneic strains differ both at H-2 and the C_H locus. As shown in Fig. 1, anti-A-gliadin responses in C.B20.B mice were significantly lower than in C.B20 and BALB/c mice.

Figure 2 compares the IgG anti-A-gliadin response in selected inbred murine strains and shows that responses in BALB/c allotype congeneic mice (C.AL20, C.B20) were >50% lower

than in the BALB/c strain. C.B20 $(H-2^a, Igh^b)$ and B10.D2 $(H-2^d, Igh^b)$ mice that share the same H-2 and Igh haplotypes, but differ markedly in background genes, responded similarly. Further, responses in C.B20.B $(H-2^b, Igh^b)$, C57BL/6 $(H-2^b, Igh^b)$ and C57BL/10 $(H-2^b, Igh^b)$ mice that share the same H-2 and Igh haplotypes were similar despite the presence of different background genes (Fig. 2).

different background genes (Fig. 2).

The BALB.B $(H-2^b, Igh^a)$ congeneic strain bears the $H-2^b$ low responder haplotype but has the BALB/c Igh^a haplotype and background genes. BALB.B mice produced significantly greater IgG anti-A-gliadin responses than other $H-2^b$ strains not bearing the Igh^a haplotype. In contrast, mice with the $H-2^b$ haplotype were low responders regardless of the Igh haplotype. Finally, BAB/14 mice $(H-2^d, Igh^{b/a})$ bear the Igh^b allotype markers of the C.B20 and C57BL strain but have some of the immunoglobulin heavy chain variable region (V_H) genes of BALB/c. BAB/14, C.B20 and BALB/c Igh congeneic mice have the same H-2 and background genes. As shown in Fig. 2, BAB/14 anti-A-gliadin responses were significantly lower than those in BALB/c mice.

These data demonstrate that genes at or closely linked to the murine C_H locus have a major role in determining the magnitude of the IgG response to A-gliadin. Thus, we have now shown two separate genetic regions that are associated with regulation of the anti-A-gliadin response—the major histocompatibility complex (H-2) on chromosome 17^{10} and the C_H locus on chromosome 12. Background genes did not significantly influence the anti-A-gliadin response in the strain combinations tested.

Anti-A-gliadin responses varied two- to threefold between H-2 identical strains bearing different Igh haplotypes. However, all $H-2^d$ strains produced significant levels of IgG anti-A-gliadin antibody irrespective of Igh haplotype, and $H-2^k$ strains behaved as low responders regardless of whether or not they bore the Igh^a haplotype. These results suggest that H-2-linked genes predominate in determining the response status to A-gliadin. Within H-2, genes mapping to the I-A subregion seem to be most important in regulating specific anti-A-gliadin responses (M.F.K., unpublished data). Products coded for by H-2- and C_H -linked genes may function independently or interact in controlling the anti-A-gliadin response.

Our data do not distinguish whether control of the anti-A-gliadin response is mediated by C_H genes or genes closely linked to that locus. Structural genes closely linked to the C_H locus are known to encode variable region idiotypic determinants on immunoglobulin heavy chains $(V_H \text{ genes})^{11-13}$. Such idiotypic

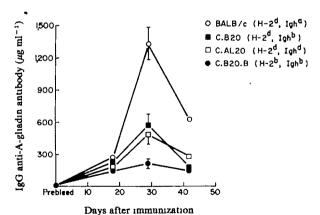


Fig. 1 IgG anti-A-gliadin responses in BALB/c allotype congeneic mice. A representative experiment is shown in which groups of four to six 10-week-old female mice were immunized with 25 μ g A-gliadin i.p. in CFA. Prebleed sera and sera obtained from individual mice on days 18, 29 and 42 after immunization were assayed for specific IgG anti-A-gliadin antibody by radioimmunoassay. Vertical bars represent s.e.m. On day 29, C.B20, C.AL20 and C.B20.B responses were significantly less (P < 0.001) than BALB/c responses, and C.B20.B responses were significantly lower than the C B20 responses (P < 0.01).

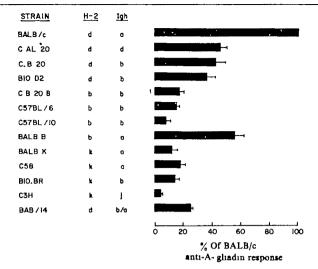


Fig. 2 Comparison of IgG anti-A-gliadin responses in inbred strains of mice. Groups of 4-12 mice of each strain were immunized with 25 μ g A-gliadin i.p. in CFA. Results compare day 29 IgG anti-A-gliadin responses in various strains with the responses in age- and sex-matched BALB/c mice that were immunized and assayed in parallel. In all strains responses on day 29 were greater than those on days 18 or 42. Anti-A-gliadin antibody was not detected in prebleed sera from any of these strains. Horizontal error bars represent s.e.m. Responses in C3H/An and C3H/St mice were the same (not shown). However, IgG anti-A-gliadin responses were 10-fold greater in C3H.OH than in either C3H strain. The C3H.OH recombinant strain is congeneic to C3H but bears the H- 2^d haplotype at K and in the I region of H-2.

determinants might regulate the anti-A-gliadin response through their involvement in lymphocyte receptor recognition of A-gliadin determinants or cellular cooperation. For example, it could be postulated that T-cell help for the IgG anti-A-gliadin response is directed to V_H -coded idiotypic determinants $^{14-18}$ on anti-A-gliadin antibody associated with the B cell. This interpretation predicts a unique pattern of idiotype expression to A-gliadin determinants among strains of mice bearing different immunoglobulin heavy chain allotypes. Alternatively, CH genes would seem to control the anti-A-gliadin response if allotype-specific T-cell help or suppression 19,20 differs among the various Igh haplotypes. However, T regulatory cells that appear to be allotype specific might be idiotype rather than allotype specific due to a close linkage and nonrandom association between C_H and V_H products²¹. Minor histocompatibility genes that are closely linked to the C_H locus²²⁻²⁴ might control the anti-Agliadin response by coding for lymphocyte cell-surface structures that are important in the recognition or binding of A-gliadin determinants, or in cellular cooperation. Finally, C_H-linked genes postulated to code for the constant regions of T-cell receptors on helper and suppressor cells25 may be important in the regulation of the anti-A-gliadin response.

To determine whether V_H genes regulate the anti-A-gliadin response, we used BAB/14 $(H-2^d, Igh^{b/a})$ mice. BAB/14 is an intra-V-region recombinant strain that bears the C_H genes of C.B20 and C57BL (that is, Igh^b) but expresses many of the V-region idiotypic markers of the BALB/c strain^{26,27}. The finding that IgG anti-A-gliadin responses in BAB/14 mice were significantly lower than in BALB/c mice could argue against V_H structural gene control of the anti-A-gliadin response. On the other hand, it is known that the BAB/14 strain expresses some C57BL idiotypes and fails to express certain BALB/c idiotypes^{26,27}. Thus, the BAB/14 data may suggest that the V_H structural genes determining the putative BALB/c A-gliadin idiotype map closer to the C_H allotype locus than to the V_H genes that determine the BALB/c idiotype to determinants like $\alpha(1,3)$ linkages on dextran B1355^{13,28}. Alternatively, differences in the anti-A-gliadin response between BAB/14 and BALB/c mice might reflect an allotype-linked regulatory

gene that controls the phenotypic expression of the putative anti-A-gliadin idiotype.

Coeliac sprue is unique among the HLA-associated diseases in that the proteins known to activate this disease are well characterized^{1,6-9}. Although more than 80% of patients in certain populations with coeliac sprue have the HLA-B8. Dw3 and DRw3 alleles2-5, many individuals with these same HLA alleles ingest dietary gluten and do not develop disease. Further, family studies indicate that HLA-identical siblings are often disparate for the development of coeliac sprue5,29. Such

Received 14 December 1981, accepted 25 January 1982.

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observations have led to the suggestion that a second genetic region may be important in the pathogenesis of this disease³⁰ Our studies with A-gliadin in mice suggest that the second genetic locus may be linked to the genes that code for immunoglobulin heavy chain allotype. Our preliminary studies in man indicate that this is indeed the case.

This work was supported by NIH grant AM 17276 and a grant from the National Foundation for Ileitis and Colitis Inc. I thank Drs D. D. Kasarda and J. E. Bernardin for A-gliadin preparations and Mr R. Austin for technical assistance.

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Radical scavenging and stimulation of prostaglandin synthesis not anti-inflammatory?

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Prostaglandins (PGs) are produced from arachidonic acid by a cascade of enzymes, the initial step being the conversion of arachidonic acid to PGG2 by cyclooxygenase. In the next step, PGG₂ is converted to PGH₂, and it is at this stage that an oxidative radical is generated, presumably OH, which is capable of inactivating several enzymes of the arachidonic acid cascade^{1,2}. This inactivation can be prevented by radical scavengers. MK 447, a phenolic compound with such properties, was indeed found to facilitate the conversion of PGG2 to PGH₂ and therefore to enhance PGE₂ synthesis^{2,3}. MK447 also inhibits irritant-induced inflammation in mice and rats; this was thought to depend on radical scavenging, which led Kuehl and colleagues to suggest that PGG2 and OH are mediators of inflammation^{2,3}. We have tested MK447 analogues containing asymmetric centres and report here that although both antipodes were equipotent radical scavengers and stimulators of prostaglandin synthesis, only the (+) antipodes had anti-inflammatory and diuretic⁴ properties. This indicates that the radical scavenging properties and pharmacological actions of MK 447 and derivatives are not interdependent and questions the pro-inflammatory role of PGG₂.

Several analogues of MK 447 were prepared and tested in a cell-free system for enhancement of prostaglandin synthesis from arachidonic acid, and in the rat for inhibition of irritantinduced paw oedema and for diuretic activity. We present here data for MK 447 and two analogues, A and B, which contain an asymmetric carbon atom (Fig. 1). Compounds A and B were resolved into their optical antipodes by crystallization of the dibenzoyl tartaric acid and tartaric acid salts, respectively, the details of which will be reported elsewhere (H.O., in preparation). The compounds were tested both as racemates and as the pure isomers.

We tested the effect of the aminophenols on the formation of PGE₂ from arachidonate by a microsomal preparation from bovine seminal vesicles. In both the presence and absence of

Table 1 Effects of MK 447 and related phenolic compounds on PGE₂ formation by bovine seminal vesicle cyclooxygenase

		Relative amounts of PGE ₂ formed		
Compound	Conc. (µM)	-GSH	+1 mM GSH	
None		100	100	
MK 447	10	159	149	
	100	323	211	
A (racemic)	10	158	148	
	100	405	236	
A (+) isomer	10	170	152	
	100	404	245	
$A^{(-)}$ isomer	10	158	152	
	100	412	223	
B (racemic)	10	187	143	
	100	391	229	
B(+) isomer	10	168	143	
	100	362	228	
$\mathbf{B}(-)$ isomer	10	184	` 144	
	100	388	241	

Bovine seminal vesicle microsomes (1.85 mg protein), prepared by the method of Takeguchi et al. 9 , were incubated with [1-Carachidonic acid (33 nmol, corresponding to 0.04 µCi) in 1 ml of 0.1 M sodium phosphate buffer pH 7.4, in the presence or absence of GSH and the indicated concentrations of test substances. After 30 min at 37 °C, the reaction was terminated by acidification to pH 4. Products and non-metabolized arachidonic acid were extracted into ethyl acetate, separated by TLC (benzene/dioxane/acetic acid = 50:50:2), eluted and measured by scintillation counting. The data are presented as percentage of controls. In control assays, PGE2 production was 217 and 1,182 ng per mg of protein per 30 min, in the absence and presence of 1 mM GSH, respectively.

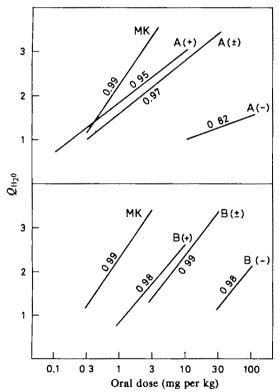
reduced glutathione (GSH), all compounds stimulated strongly the formation of PGE₂ (Table 1) and PGF₂ (not shown) in a concentration-dependent manner. However, in the presence of GSH, which by itself stimulates PGE₂ formation⁵, the enhancement by the phenolic compounds was reduced. Independent of their structure and chirality, all compounds tested showed similar effects. The same results were obtained for various other phenolic compounds (data not shown). In agreement with Kuehl et al.3, the structural determinants essential for activity were found to be the phenolic hydroxyl group and an adjacent aminomethyl substituent.

The anti-oedematous and diuretic effects of MK 447 and compounds A and B are summarized in Table 2. MK 447 and

Fig. 1 Structure of MK 447 and the two optically active derivatives, compounds A and B.

both racemates of the two analogues showed moderate to strong inhibition of the inflammatory response induced by intraplantar injection of carrageenin, and markedly enhanced water and sodium excretion. Despite their close structural relationship and almost identical stimulation of prostaglandin synthesis, compounds A and B were only about one third and one tenth, respectively, as active as MK 447 in inhibiting paw oedema and enhancing diuresis. Most remarkably, both in vivo activities appeared to be due to the (+) isomer. As shown in Fig. 2, the (-) isomers are both much less effective than the (+)isomers or the racemates.

Comparison of the pharmacological activities of the two isomers is only valid if their absorption and fate in vivo are similar. This was tested in the case of compound B. The (+) and (-) antipodes were administered orally to separate groups of rats at a dose of 10 mg per kg, and urine was collected during the following 7 hours. In analogy with MK 447 (ref. 6), Osulphate was expected to be the major urine metabolite. The urine was desalted using Sep-pak cartridges (Waters Associates, Massachusetts) and incubated at 55 °C for 2 h with Helix pomatia extract (100,000 Fishman units of β -glucuronidase and 10° Roi units of arylsulphatase). The samples were then evaporated to dryness, taken up in a small volume of ethanol



r(

Fig. 2 Dose-dependent diuretic effect of MK 447 and related phenolic compounds in the rat (for method see Table 2), expressed as the ratio of water excretion in the treated compared with that in control animals, $Q_{\rm H2O}$. The curves were calculated by regression analysis of the values obtained for individual animals; the correlation coefficient, r, is shown for each curve. Each compound was tested at 3-5 doses in 3-4 animals per dose. MK, MK 447.

Table 2 Anti-oedematous and diuretic activities of MK 447 and related phenolic compounds in the rat

	Inhibition of carrageenin and naphthoylheparamine-induced paw oedema (ED 50; mg per kg p.o.)		Diureti in th (ED ₁₀₀ ; mg Water	e rat
Compound	C C	NH	excretion	excretion
MK 447	3.9	2.8	0.7	0.75
A (racemic)	10.6	13.2	2.0	1.5
A (+) isomer	6.5	8.2	1.1	1.1
A (-) isomer	>30.0	>30.0	>100.0	>100.0
B (racemic)	12.2	14.2	6.5	7.3
B(+) isomer	4.3	4.0	4.6	4.5
B(-) isomer	>30.0	>30.0	85.0	>100.0

The carrageenin (C) oedema test 10 and the naphthoylheparamine (NH) oedema test¹¹ were performed in the rat according to established methods. In both cases, paw swelling was assessed by conductometry as described by Kemper and Ameln¹². The compounds were given orally (p.o.) as a suspension in saline containing 0.5% tragacanth, 1 h before local injection of the irritant. The ED₅₀ (the average dose giving 50% inhibition of irritant-induced swelling compared with untreated controls) was determined from dose-response curves (3-4 doses per compound, 5 rats per dose). The inhibition of swelling after a dose of 30 mg per kg of the (-) isomers was 15 and 2% for compound A and 19 and 7% for compound B. Diuretic activity was assessed by the method of Flückiger et al. 13. Fasted rats (200 g) were hydrated (30 ml water per kg, orally) and 2 h later, after emptying the bladder, they were given the test compounds in 50 ml of isotonic saline per kg. The total urine produced during the following 3 h was measured and tested for sodium, potassium and chloride content. Average water and sodium excretion per 200 g body weight was calculated (3-4 rats per dose) and the ED₁₀₀, the average dose inducing a 100% increase in both values, determined for 3-5 different dosages.

and analysed by TLC. Three solvent systems were used and the compounds were detected under UV light and with iodine vapours. Similar amounts of the aminophenol were obtained after administration of either antipode, indicating that they were absorbed and excreted to the same extent. In both cases, no aminophenol was detectable in urine samples which were not treated with the hydrolases, suggesting that the metabolism of the two antipodes was similar.

Our results show that the anti-oedematous effects of phenols like MK 447 and its analogues are unlikely to depend on the ability of the compounds to scavenge oxygen radicals and hence to increase the conversion of PGG₂ to PGH₂ and further metabolites. The two optical isomers of compounds A and B enhance prostaglandin synthesis to the same extent. As indicated by the experiments with compound B, both isomers have similar absorption and fate in rats. Nevertheless, they differ widely in their in vivo activities, the (+) isomers only being biologically comparable to MK 447. These results suggest that the theory that radicals which arise from the peroxidase-dependent reduction of PGG₂ are pro-inflammatory and that PGG₂ is a key element in the pathogenesis of inflammation^{2,3,7} needs revision.

Our results also question the notion that the main action of MK 447, that is, diuresis and saluresis⁴, is mediated by its enhancing prostaglandin synthesis. This was suggested by the observation that MK 447-induced diuresis was inhibited by indomethacin^{4,8}. However, as the (-) isomers of compounds A and B also have some diuretic activity, it cannot be ruled out entirely that an increase of prostaglandin production may contribute to the overall effect.

Received 8 December 1981, accepted 21 January 1982

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A model of the nerve impulse using two first-order differential equations

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The Hodgkin-Huxley model of the nerve impulse consists of four coupled nonlinear differential equations, six functions and seven constants. Because of the complexity of these equations and the necessity for numerical solution, it is difficult to use them in simulations of interactions in small neural networks. Thus, it would be useful to have a second-order differential equation which predicted correctly properties such as the frequency-current relationship. Fitzhugh² introduced a secondorder model of the nerve impulse, but his equations predict an action potential duration which is similar to the inter-spike interval3 and they do not give a reasonable frequency-current relationship. To develop a second-order model having few parameters but which does not have these disadvantages, we have generalized the second-order Fltzhugh equations2, and based the form of the functions in the new equations on voltageclamp data obtained from a snall neurone. We report here an unexpected property of the resulting equations—the x and y null clines in the phase plane lie close together when the phase point is on the recovery side of the phase plane. The resulting slow movement along the phase path gives a long inter-spike interval, a property not shown clearly by previous models² The model also predicts the linearity of the frequency-current relationship, and may be useful for studying detailed interactions in networks containing small numbers of neurones.

Although our model may be seen as a generalization of the Fitzhugh equations², it can be developed from first principles if it is assumed that the rate of change of membrane potential depends linearly on z (the current passed through the electrode), and y (an intrinsic current), and depends nonlinearly on membrane potential, giving

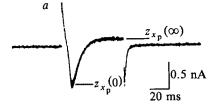
$$\dot{x} = -a(f(x) - y - z) \tag{1}$$

We assume also that the rate of change of intrinsic current is given by

$$\dot{y} = b(g(x) - y) \tag{2}$$

In these equations, a and b are constants, and equation (2) has a form which ensures that the time course of z in voltage-clamp conditions is exponential (see equation (4)). This is a simplification which ignores the voltage-dependence of the time

To determine the form of f(x) and g(x) in equations (1) and (2), a large cell from the visceral ganglion of the pond snail, Lymnaea siagnalis, was clamped to a range of different voltages (x_p) , and the initial $(z_{x_p}(0))$ and steady-state $(z_{x_p}(\infty))$ values of the clamping current were measured, giving a conventional⁵ current-voltage plot (Fig. 1). As the cell fires repetitively in the resting state, the membrane potential was biased initially by adjusting z so that the cell just stopped firing. This corresponds to an artificial stable equilibrium point, and will be used as the origin (x = 0, y = 0) in the phase plane. In the current-voltage plot (Fig. 1), the scale is also positioned so that the current required to maintain the equilibrium voltage is at the origin



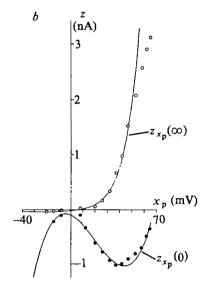


Fig. 1 Voltage-clamp data on which the model is based. In these experiments, the brain of the pond snail L. stagnalis was pinned out in a chamber with a sylgard base, and the outer sheath softened using pronase enzyme for a few minutes before recording. The bathing solution had the following composition (mM): Na⁺, 24.0; K^+ , 2.0; Ca^{2+} , 4.0; Mg^{2+} , 2.0; Cl^- , 38.0; $H_2PO_4^{2-}$, 0.1; Na-HEPES, 35.4. A large cell (~200 μ m) in the visceral ganglion was impaled with a bevelled glass microelectrode filled with 3 M KCl, and having a resistance of 4-5 MΩ. The cell was clamped using the single-electrode voltage-clamp technique of Wilson and Goldner', a. Current change recorded for a voltage step (x_n) of 25 mV from the equilibrium point (cell held at an actual potential of -60 mV, which is the equilibrium point, x = 0, in the measurements). The initial $(z_{xp}(0)$, or peak inward) and final $(z_{xp}(\infty)$, or steady-state outward) currents are indicated, the final currents being measured after 60 ms in this case. b, Initial and final currents obtained from records such as shown in a are plotted for different values of clamp potential (x_p) measured from the equilibrium point. Negative values of z are inward, and positive values are outward currents. Final currents were measured 100 ms after onset of voltage step. Different animal than in a, but curves are typical for this cell (experiment repeated five times). The $z_{xp}(0)$ points have been fitted by the least-squares method, using the cubic equation $f(x) = cx^3 + dx^2 + ex + h$, where c = 0.0001, d = 0.001, e = 0.01 and h = 0.1. The $z_{x_p}(\infty)$ points were fitted by an exponential of the form $g(x) = q e^{rx} - s$, where q = 0.024, r = 0.088 and s = 0.046.

(z=0). Therefore, at the equilibrium position $x=y=\dot{x}=\dot{y}=$ z = 0. The time course of the current, after the onset of a step in voltage to x_p , is given (from equation (1) with $\dot{x} = 0$) by

$$z_{x_p}(t) = f(x_p) - y(t) \tag{3}$$

where y satisfies equation (2) with $x = x_p$. Therefore, $z_{x_0}(t)$ is given by

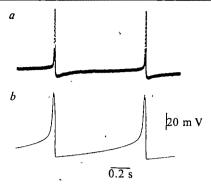
$$z_{x_0}(t) = f(x_0) - g(x_0)(1 - e^{-bt})$$
 (4)

and hence

$$z_{x_p}(0) = f(x_p), \text{ and } z_{x_p}(\infty) = f(x_p) - g(x_p)$$
 (5)

To obtain $f(x_0)$, the values of the initial currents $(z_{r_0}(0); \text{ Fig.})$ 1b) were fitted by a cubic equation. To obtain $g(x_p)$, an exponential function was fitted to the value of the final currents $(z_{xx}(\infty))$; Fig. 1b), and $g(x_p)$ was obtained from equation (5). The time constant b was estimated from voltage-clamp current records

Fig. 2 Comparison between a, actual and b. calculated waveforms for the same cell as in Fig. 1b. The calculated waveform is the solution to equations (7) and (8) for a current of z =0.008, with the constants $a = 5,400 \text{ mV s}^{-1}$ nA^{-1} , $b = 30 \text{ s}^{-1}$ and and using the functions f(x)and g(x) shown in Fig. 1b. Numerical solutions were obtained using the



Runge-Kutta-Merson method⁸ with variable step length. In these calculations we found that repetitive firing occurred down to z=-0.026 rather than z=0 as expected (procedure outlined in the text). This was due to the approximations adopted, which slightly changed the values of f(x) and g(x) at the origin. As the computed equilibrium point is at z=0.034 (=0.008±0.026) in Fig. 4. The recorded action potentials were obtained in current-clamp conditions with an experimental z value of 0.04 and were photographed ~ 1 min after imposing the current step.

(Fig. 1a), which were assumed to follow an exponential time course, and the time constant a was obtained by applying a current step to the system at rest in the equilibrium position (0,0). If the step in current is $z(t) = z_p$ for $t \ge 0$ then a may be obtained from the slope of the voltage change immediately after the onset of the current-clamp step. This is given by equation (1) with f(0) = 0 and y(0) = 0:

$$\dot{x}(0) = az_{p} \tag{6}$$

Thus the assumed form for our equations is

$$\dot{\mathbf{x}} = -a(f(\mathbf{x}) - \mathbf{y} - \mathbf{z}) \tag{7}$$

$$\dot{y} = b(f(x) - q e^{rx} + s - y) \tag{8}$$

where $f(x) = cx^3 + dx^2 + ex + h$, and a-h, q, r and s are constants. After measuring a and b, and fitting cubic and exponential functions to the $z_{x_p}(0)$ and $z_{x_p}(\infty)$ data of Fig. 1b, the solutions of equations (7) and (8) were obtained by numerical integration.

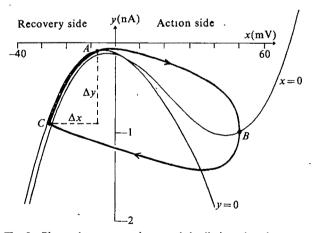


Fig. 3 Phase plane representation of the limit cycle solution to equations (7) and (8). The values of the constants are the same as in Fig. 2, except that a z value of 0.033 has been used so that the equilibrium point can be more clearly distinguished. The x=0 curve has been displaced horizontally to the right by 2-3 mV so that the separation between the x and y null clines on the recovery side of the diagram can be seen more easily. The x=0 curve is similar to that of Fitzhugh², but the y=0 curve bends round to follow the x=0 curve in the lower left-hand quadrant, which is significantly different from the Fitzhugh model. The limit cycle is divided into an action potential phase $(A \to B \to C)$ and a recovery phase $(C \to A)$, during which the phase point moves slowly between the x and y null clines. The differences Δx and Δy are, respectively, the changes in membrane potential and intrinsic current during recovery.

Figure 2 compares the calculated x(t) waveform with that recorded from the cell. The model clearly predicts a ratio of spike duration to inter-spike interval which is similar to that actually recorded, although the calculated action potential is of rather longer duration than the recorded spike; the pacemaker potential also rises to a higher threshold in the model. Some of these differences may be corrected by taking into account the voltage dependency of the time constants, but these modifications are not introduced here because they detract from the simplicity of the model.

Although this model is not as accurate as more complicated models of repetitive firing⁶, it has a conceptual advantage which becomes apparent when the oscillation is plotted onto the phase plane. The main difference between this model and that of Fitzhugh² is the introduction of g(x) into the equation for \dot{y} . By using the phase diagram (Fig. 3) it becomes easy to understand why this modification is so important in lengthening the recovery phase between successive action potentials. In the phase plane, when the limit cycle crosses the $\dot{x} = 0$ curve at C (Fig. 3), it is constrained to move along the narrow path between the $\dot{x} = 0$ and $\dot{y} = 0$ curves. Because the phase path is so close to both the x and y null clines, progress along the limit cycle will be much slower from C to A (the pacemaker or recovery phase) than from A through B to C (the action potential part of the cycle). By contrast, in Fitzhugh's model the $\dot{x} = 0$ and $\dot{y} = 0$ curves diverge markedly on both sides of the diagram, so that x and y change almost as rapidly during the pacemaker phase as during the spike itself.

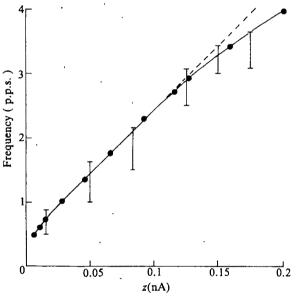


Fig. 4 Comparison of the calculated and experimental frequency-current relationship (pulses per second). The calculated frequencies () were derived from numerical integration of equations (7) and (8) using the same parameter values as in Fig. 2 but with different values of z Calculated frequencies are plotted relative to the computed equilibrium point (z = -0.026; see Fig. 2). Experimental z values were measured relative to the experimental equilibrium point (at which z=0; there were no action potentials). Over most of the range there is a linear relationship between frequency and applied current, with both experimental and calculated curves having a similar slope. At higher values of z, the calculated curve and the experimental points begin to approach a limiting frequency at a similar z value, although the experimental points appear to fall off more sharply from linearity (broken line) than the theoretical prediction. This particular cell also shows adaptation of firing frequency to an applied current step, so that the values for the experimental frequencies lie within the upper (-initial frequency in response to step) and lower (=final frequency) limits of the bar lines. Thus a precise comparison cannot be made because the model has not yet been modified to include adaptation. Experimental points were obtained from a different animal than that used in Fig. 1b, but the experiment was repeated four times and gave consistent results.

Numerical solution may also be used to predict the frequency-current relationship. The computed values of the frequency are linearly related to the current (Fig. 4) and compare reasonably well with the experimental values. It is also possible to derive an expression for the frequency-current relationship fairly simply. From equations (1) and (2) we see that

$$b\dot{x} + a\dot{y} = ab(g(x) - f(x)) + abz \tag{9}$$

Then the expression g(x)-f(x) (= $-z(\infty)$; equation (5)) may be approximated for x < 0 by a small positive constant M (see $z_x(\infty)$ in Fig. 1b). Integrating (9) we obtain

$$b\Delta x + a\Delta y = abMT + abzT \tag{10}$$

where Δx and Δy are the changes in the values of x and y during recovery (from C to A in Fig. 3), which occurs in time T, say. If we ignore the spike duration, the frequency f of oscillation is given by

$$f = \frac{1}{T} = \frac{ab(z+M)}{b\Delta x + a\Delta y} \tag{11}$$

from which we can see that, to the extent that Δx and Δy are independent of z, f is linearly related to z (see Fig. 4).

The model therefore predicts the frequency-current relationship as well as giving a reasonably accurate representation of the time course of the membrane potential change. It also provides a simple explanation of why the recovery time is much longer than the spike duration. Because these equations are simpler to understand than the four-dimensional Hodgkin-Huxley equations¹, and can be solved numerically in a shorter time, they may be useful in studying small neural networks.

We thank Dr W. A. Wilson for supplying us with further information on the single electrode voltage-clamp, and Mr W. L. Barry and Mr T. Ford for constructing the voltage-clamp apparatus.

Received 1 September 1981, accepted 26 January 1982

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Induction of helical liposomes by Ca²⁺-mediated intermembrane binding

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Extensive freeze-fracture electron microscopic studies of Ca2+containing mixtures of an acidic phospholipid (cardiolipin) and phosphatidylcholines¹⁻⁵, have revealed certain replica features which have been interpreted as regions of membranemembrane attachment³⁻⁵. Spin-label paramagnetic resonance spectra of mixtures of another acidic phospholipid (phosphatidylserine) and phosphatidylcholines have provided evidence for Ca2+-mediated lateral phase separations of the acidic phospholipids⁶⁻⁸, and their involvement in intermembrane binding⁷. We report here that in the presence of Ca²⁺ binary mixtures of cardiolipin and phosphatidylcholines readily form tightly folded single- and double-helical liposomes; we invoke membrane-membrane attachment to explain this phenomenon.

Ethanolic solutions of bovine heart cardiolipin (Ca²⁺-free: Sigma) and dimyristovlphosphatidylcholine (Sigma) were evaporated to dryness to form binary mixtures containing 37 mol % cardiolipin (1.7 mg total lipid). The lipid was dissolved in chloroform/methanol (10:1 v/v) and evaporated to dryness, then 160 µl phosphate-buffered saline (PBS) were added with or without 2 mM EDTA and the mixture was incubated at 45 °C for 5 min, vortexed for 10-15 s, then diluted 20-fold with PBS. All experiments were carried out at 25-26 °C. well above the chain-melting transition of dimyristoylphosphatidylcholine (23 °C).

We used two experimental protocols: (1) after addition of PBS+EDTA and vortexing, the dilution with PBS contained no Ca²⁺. The sample was placed on a microscope slide with an elevated coverslip (100 $\mu m)$ and a concentrated CaCl $_2$ solution (0.01-1 M) was added from one side. Ca2+ ions gradually diffused through the solution, allowing observation of helix formation (see Fig. 1a-d). (2) For the mixture incubated without EDTA, the subsequent dilution with PBS contained various low concentrations of Ca^{2+} ($10^{-6}-10^{-3}$ M).

Single and double helix formation, observed using protocol (1), is shown in Fig. 1. Long tubes were often observed in the absence of Ca²⁺, together with liposomes having typical irregular shapes. The presence of regular tubes and irregular liposomes may be explained by possible lipid compound formation and phase separations in binary mixtures of these lipids9. The tubes were almost perfect cylinders, although end defects were frequently seen, and were usually multilamellar but occasionally unilamellar, as judged by microscopic observation of pronounced brownian motion, characteristic of unilamellar liposomes¹⁰. Photobleaching recovery experiments measuring diffusion parallel to the long axis of the tubes yielded lateral diffusion coefficients typical of fluorescent lipid probes ($D \sim$ $10^{-8} \, \text{cm}^2 \, \text{s}^{-1}$).

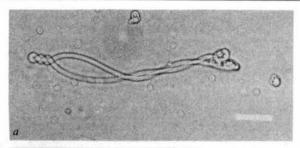
Protocol (1) provided evidence that the rate of helix formation increased with increasing Ca²⁺ concentration. Protocol (2) permitted the determination of the minimum concentration of tion required for helix formation; compared with controls (no Ca²⁺), significantly more helices were formed at Ca²⁺ concentrations as low as 10^{-6} M.

The thermodynamic driving force for helix formation is the Ca2+-mediated membrane-membrane binding. At higher Ca2 concentrations, helices rapidly collapse on themselves forming a variety of more complex structures. It is possible that helices represent kinetic intermediates in the Ca2+-mediated transformation of tubes to more complex structures, as each step of helix formation represents an increase in the area of membranemembrane contact. The formation of a typical single helix is simple; it is initiated at one end, and is smooth and continuous. However, double-helix formation is sometimes complex; it initiates from a hairpin loop and progresses in a stepwise manner. A complete regular double helix with no loose ends can initiate at a hairpin loop but not in the centre of a tube; this requires sliding as well as twisting of one tube relative to the other (see Fig. 1a-c).

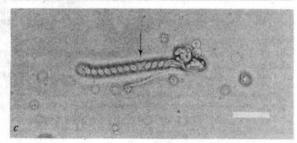
The rate at which helices form in the presence of Ca²⁺ is consistent with earlier proposals concerning Ca2+-mediated lateral phase separations of acidic phospholipids⁶⁻⁸, and with the rates of lateral diffusion of these lipid molecules reported here. Ca²⁺ chelation by phosphate groups should be favoured when these groups have a three-dimensional configuration, rather than a two-dimensional configuration provided by a single membrane.

The forces opposing helix formation are weak. The shear modulus of a fluid membrane is essentially zero; a negligible force is required to twist one region of a lipid tube relative to another. We observed no distortion of the circularity of the tubes, thus there was no detectable volume or surface area change of the tubes on helix formation. Note that the outer diameter of the double helix in Fig. 1 is twice the diameter of the tube. No stretch or compression elastic energy is required to convert a cylindrical tube into a helix because lipids on the

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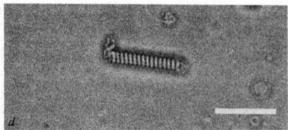


Fig. 1 Helical liposomes derived from binary mixtures containing 37 mol % cardiolipin and 63 mol % dimyristoylphosphatidylcholine as Ca2+ ions diffuse from a 0.01 M CaCl2 solution at the edge of a coverslip into the region where the liposomes are located. a-c Illustrate the formation of a double helix initiated on the left at a hairpin loop, and on the right in an extended region of membrane-membrane contact. In a and b both helices are righthanded. In subsequent photographs (not shown) the helix at the right-hand end became unwound as the helix on the left continued to form. Subsequently, as the right-handed helix to the left continued to form, a left-handed helix formed on the right, and the two helices met in a soliton-like defect indicated by the arrow in c. A single helix is shown in d. Scale bars, 25 µm. The elapsed time from a to c is \sim 5 min; double-helix formation is not continuous but proceeds in a stepwise fashion. A similar length of time was observed for the continuous formation of the single helix shown completed in d.

inside of the helix can flow to the outside on bending. Helfrich¹¹ has estimated the curvature elastic energy for bending a lipid tube (of radius r) so that it has a radius of curvature R: to first-order approximation this is $\pi Kr/2R^2$ per unit length, where K is the curvature elastic modulus. For egg lecithin, $K \simeq$ 23×10^{-12} erg (ref. 11). Assuming one lipid molecule per 10 Å along a contact line between helical tubes, and using the above values of K, we calculate the curvature elastic energy of lipid touching this line to be 0.013n kcal mol⁻¹ for a tube with radius $r = 1 \mu m$ and having n bilayer membranes. Thermal energy, RT, at room temperature is 0.6 kcal mol⁻¹. Clearly, weak membrane-membrane binding energies can overcome curvature elastic energy and stabilize helices.

We acknowledge that Dr Alec Bangham has independently observed the formation of tubular and helical liposomes on

hydration of egg lecithin with saline solution, and we thank him for supplying photographs of these helical liposomes. The double helices of egg lecithin are very similar to those reported here. This work was supported by NSF grant PCM 8021993.

Received 23 November 1981; accepted 21 January 1982.

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Effects of Duchenne muscular dystrophy on muscle protein synthesis

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The primary defect in Duchenne muscular dystrophy (DMD) is unknown but muscle growth failure and wasting of skeletal muscle must be the result of an imbalance between protein synthesis and degradation, that is, the rate of muscle protein synthesis is insufficient to replace degraded proteins and expand the protein mass. In patients with DMD, excretion of 3-methylhistidine is dramatically increased relative to creatinine and this is commonly accepted as evidence of increased muscle myofibrillar degradation¹⁻³. As rapid wasting of muscle has not been observed to occur in the disease, increased protein degradation ought to be accompanied by increased protein synthesis and this has been observed or implied from measurements in animal models of the disease4-6. However, so far no direct measurements of muscle protein synthesis or degradation have been made in patients. We report here the first direct measurements in vivo of rates of skeletal muscle protein synthesis in patients suffering from DMD. The results, obtained using stable isotope tracers, clearly show that a marked reduction in muscle protein synthesis is the primary cause of the growth failure and that muscle protein degradation cannot be elevated.

We studied seven normal fed adult men aged 22-65 yr and five boys with Duchenne muscular dystrophy, aged 12-18 yr. Three of the patients were wheelchair bound and the two youngest were on the verge of becoming so. Investigations of these subjects using radioactive isotopes would not have been ethically feasible. We infused [1-13C]leucine intravenously after a priming dose, and measured the incorporation of labelled leucine into skeletal muscle proteins in samples obtained by needle biopsy8 (Fig. 1). This approach also allows the measurements of whole-body leucine flux and its components, leucine oxidation and whole-body protein synthesis (Table 1)

The rate of muscle protein synthesis in normal adults was determined from the increase in isotopic enrichment between two biopsies; as synthesis rates calculated from the enrichment observed in a single biopsy taken after 7 h were not significantly different from this estimate, we calculated synthesis rates in patients from the enrichment in a single biopsy sample taken at 7.5 h. The precursor labelling was assumed to be that of the keto acid of leucine, α -ketoisocaproate (α -KIC), in plasma. Most plasma KIC originates from skeletal muscle¹⁰ so that its enrichment should closely reflect that of intracellular leucine in muscle. This was confirmed in four normal adults and three patients by direct measurements of the enrichment of free leucine in the muscle biopsy intracellular water (unpublished results).

In normal adults (Table 1) the rate of muscle protein synthesis was 0.198% h⁻¹, twice the value determined previously¹¹. However, in those studies using ¹⁵N-lysine, the precursor labelling was taken as that of plasma lysine, which we believe gave erroneous results. Studies using infusions of lysine and leucine in pigs have shown that the ratio of intramuscular free lysine labelling relative to plasma is less than 50% of that for leucine ¹², which explains the discrepancy and supports our current measurements. From creatinine excretion as a proportion of muscle mass¹³ and from our measurements of muscle protein content (17%) and leucine content of protein (8%), we estimate that in a man weighing 70 kg, 126 g of protein are synthesized per 12 h in the fed state, that is 53% of the whole-body synthesis rate. This is a higher proportion than that observed in smaller animals¹⁴.

In the patients with DMD, whole-body protein synthesis was depressed as was that in muscle (Table 1), the former being only half that observed in normal adults, and as all dietary leucine was oxidized, no net protein deposition could occur. The rate of muscle protein synthesis measured during feeding

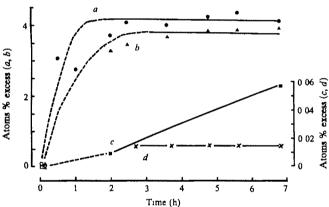


Fig. 1 Enrichment with 13 C of blood leucine (\bullet) and of α -KIC (\blacktriangle) (transamination product of leucine), leucine incorporated into quadriceps muscle protein (a) (obtained by needle biopsy, with local anaesthesia) and of expired CO₂ (x) during the primed (0 8 mg per kg) infusion (1 mg per kg per h) of [1-13C]leucine (KOR Inc., Massachusetts) in a normal healthy adult man (80 kg, 42 yr old). A similar pattern of changes was observed in all subjects. The subject was fed 0.1 g protein and 14 kJ per kg at hourly intervals. Blood was sampled via an indwelling cannula and the enrichment with 13 C of leucine and α -KIC determined by gas chromatography—mass spectrometry^{7,30}. Expired CO₂ was collected in 5-1 Douglas bags attached to a three-way valve and mouthpiece and a portion of the sample immediately transferred to an evacuated glass vial. The enrichment of CO₂ with ¹³C was measured in a VG micromass 602D motope ratio mass spectrometer after drying and trapping of solid CO2 in a cold-finger cooled with liquid N₂ (ref. 31). Total CO₂ production was measured from the IR absorbance of expired air sampled continuously from a metabolic tent, or collected over 5 min in 100-l Douglas bags Alkalisoluble muscle protein from fat-free, dried biopsy samples was hydrolysed and leucine was separated preparatively on a Locarte amino acid analyser Leucine-CO2 was liberated by ninhydrin at pH 2, dried and its enrichment measured in the isotope ratio mass spectrometer. Muscle protein synthesis, k_{μ} may be obtained by comparing the change in ¹³C enrichment of leucine incorporated into protein in two muscle biopsy samples taken some hours apart, but at times (t_1, t_2) when the blood leucine or α -KIC pools are enriched to plateau values. Synthesis rate, $k_a = \Delta E_{\rm muscle}/\Sigma_1^{r_1} E_{\rm blood} \Delta l \times 100 \; {\rm h}^{-1}$ where $\Delta E_{\rm muscle}$ is the change in enrichment of muscle and $\Sigma E_{\rm blood}$ is the integral of blood over the time in h. Note in the figure that with an assumed initial zero (that is, background) enrichment of muscle-protein leucine (as measured in muscle from surgical patients, in mixed blood proteins and in casein), incorporation of ¹³C into muscle protein is essentially linear with time. There is thus little error in calculating synthesis on the basis of either the change in enrichment between the biopsies compared with the plateau of blood leucine or α -KIC enrichment, or the incorporation of ¹³C-leucine into muscle obtained by a single biopsy at the end of the whole period of infusion, compared with the integrated weighted-mean blood leucine (or α -KIC).

Table 1 Skeletal muscle and whole-body protein synthesis in normal adults and boys with Duchenne muscular dystrophy

Whole-body protein turnover	Normal adults $(n = 7)$	Boys with DMD $(n=5)$
Body weight (kg)	80.8 ± 19.2	54±6.5*
Leucine flux Q	194 ± 20	140±39
(µmol per kg per h)	17.1-20	1.0207
Leucine intake I	55±4	64 ± 10
(μmol per kg per h)		**
Leucine oxidation O	40±8	$63 \pm 30*$
(µmol per kg per h)		
Protein synthesis S	154 ± 30	$77 \pm 22*$
(µmol leucine per kg per h)		
(g protein per 12 h)	212 ± 41	81 7 ± 23*
Muscle protein synthesis		
Synthesis rate (% h ⁻¹)	0.198 ± 0.055	0.066 ± 0.028 *
Muscle mass (kg)	31.1 ± 7.7	$6.4 \pm 3.0*$
Muscle protein synthesis (g per 12 h)	113 ± 31	17 5±7.42*
Urinary 3-methylhistidine/creatinine (molar ratio×10 ³)	19.0 ± 2.4	55.2±5.4*

Data are mean ±s.d. for volunteers, consisting of seven healthy adult males (aged 22-65 yr) and five children with Duchenne muscular dystrophy, aged 12-18 yr. Subjects were infused intravenously with carboxyl-labelled ¹³C-leucine (enriched to 90 atoms % excess) for 7-8 h of the study while being fed a liquid milk-based food at hourly intervals (~0.1 g, protein, 14 kJ per kg body wt) Leucine flux and oxidation were calculated using equations given in ref. 7, except that the enrichment of blood α -ketoisocaproate rather than leucine was used as this is more likely to represent the labelling of the metabolic pool from which leucine is removed by protein synthesis and oxidation. The component of the flux not directly measured, protein synthesis (S), was obtained by manipulation of the data for flux (Q) and oxidation rate (Q), according to a two-pool stochastic model whereby in the steady state $\dot{Q} = \dot{S} + \dot{O}$ (that is, the sum of processes removing leucine)⁶. (See refs 7, 30, 31 for details of mass spectrometric analysis) Muscle synthesis rate was measured by ¹³C incorporation into leucine of mixed muscle protein of quadriceps (see Fig. 1) assuming that the precursor pool for protein synthesis was labelled similarly to a-KIC in blood. Urinary 3-methylhistidine and creatinine were measured in most normal subjects over 24 h on various occasions but were also measured during infusion of labelled leucine. In the case of the patients with muscle disease, values for urmary 3-methylhistidine and creatinine are the mean of estimates obtained over a number of days. There were no significant differences in the mean values of such estimates compared with those obtained during the period of infusion. Muscle mass was estimated from creatinine excretion (8 84 mmol of creatinine = 20 kg of muscle)13

* Significant difference (P < 0.01; Student's one-tailed t-test) from levels in normal fed men

was only 34% of that observed in the normal adults (that is, $0.066\%\ h^{-1}$), which is less than that observed during fasting in normal adults (unpublished results). This means that muscle protein synthesis contributes only 17.5 g protein per 12 h, 8.6% of whole-body protein synthesis. This depression of muscle protein synthesis is all the more marked when it is considered that in normal adolescent children who are still growing, the rates of whole-body and muscle protein synthesis are likely to be much higher than in adults.

The finding of a depressed rate of muscle protein synthesis associated with DMD was unexpected, particularly in view of the high 3-methylhistidine/creatinine ratio observed in the present patients and reported previously in other patients with this disease¹⁻³. 3-Methylhistidine occurs in actin and some species of myosin heavy chain, and as it is not metabolized in man its excretion should be a quantitative estimate of the rate of actin and myosin heavy chain degradation¹⁵. Creatinine excretion may be taken as an index of skeletal muscle mass¹³ (even in cases of muscle disease¹⁶); thus the threefold elevated 3-methylhistidine/creatinine ratio observed in boys with DMD has been taken to indicate a proportionally elevated rate of mvofibrillar degradation¹⁻³. In addition, as these patients were not actually losing muscle protein at a significant rate (on the basis of our repeated computerized tomography and creatinne excretion measurements), the rate of protein synthesis should therefore be increased compared with normal values. Clearly this conclusion does not agree with the rates of muscle protein synthesis measured here, and have no reason to believe that our direct measurements of the rate of muscle protein synthesis are substantially wrong. For this to be the case, enrichment of the true precursor leucine pool would have to be very much lower than the α -KIC enrichment in the patients compared

with the normal adult subjects, which is unlikely. On the other hand, we have already reported findings that raise doubts about the validity of the assumption that 3-methylhistidine excretion rates are a true reflection of skeletal muscle degradation rates 17-19. We have shown that in the rat, substantial amounts of 3-methylhistidine may derive from the degradation of actin in non-muscle tissues. Although the amounts of actin in tissues other than skeletal muscle is small, it is a ubiquitous protein and if its turnover rate in non-muscle tissues is high this could result in a substantial non-muscle contribution to the urine.

In fact the proportion of non-muscle sources of excreted 3-methylhistidine is likely to be much higher in the dystrophic patients than in normal subjects. We find, on the basis of creatinine excretion, body weight and total body potassium measurements, that although skeletal muscle mass is markedly reduced in these patients (to only 12% of body weight), nonskeletal muscle mass (normally 15% of body weight) is only reduced to 74% of normal. If creatinine excretion is depressed in proportion to the reduced muscle mass, non-muscle sources of 3-methylhistidine would inflate the 3-methylhistidine/ creatinine ratio, as has been observed in muscle waiting due to amyopathic lateral sclerosis²⁰. Given the 3-methylhistidine content of skin (0.6 µmol per g protein) and gastrointestinal muscle (1.8 µmol per g protein)²¹ and assuming that these two tissues comprise a normal proportion (47 and 13% respectively)22,23 of non-skeletal muscle tissues in patients with muscular dystrophy, then these tissues need only turn over at three to four times the rate of skeletal muscle to account for the excreted 3-methylhistidine. Studies of patients undergoing elective surgery have provided evidence that in man, skin and gut muscle do indeed turn over at about two and five times, respectively, the rate of skeletal muscle²⁴.

An elevated rate of protein degradation in diseased muscle has also been observed in animal models of the disease However, there is no animal model which reproduces exactly the features of the human disease. Indeed, in most of the animal models there is a transient muscle hypertrophy early on in the disease, and protein turnover measurements are most often made in animals at this stage. As we have pointed out elsewhere²⁵, increased protein turnover in muscle is often a feature of rapid growth and regeneration, which may account for the elevated turnover in these animal models26. There is no evidence to suggest that this occurs in adolescent boys with muscular dystrophy such as those studied here, although it may occur in very much younger patients with the disease.

A reduced rate of protein synthesis rather than an increased rate of protein degradation as the primary cause of growth failure in DMD has important implications for present disease therapies. The notion that increased degradation was the main factor led to suggestions (and testing in animal models) that proteinase inhibitors might prove effective. 27-29. However, suppression of muscle protein degradation falsely assumed to be elevated in patients would be of little value since protein synthesis is markedly depressed. The use of possible therapeutic agents to increase muscle protein synthesis would be much more rational. The present results demonstrate that it would be possible to monitor such effects over the short term.

This work was supported by grants from the Muscular Dystrophy Group of Great Britain, Muscular Dystrophy Association of America, the British MC, Wellcome Trust, Action Research for the Crippled Child, Abbott Laboratories, and NIH grants AM-25994 and RR-00954. S.L.W. was a Canadian MRC Fellow. We thank the dietitians and nursing staff of the Hospital for Tropical Disease and University College Hospital for their assistance and the staff of the London Broadcasting Company's A.M. programme who helped us locate lost samples. We also thank M. A. Read, W. W. Read, M. Nathan, P. C. Bates and A. Mellone for technical assistance.

Received 14 October 1981, accepted 26 January 1982

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High-affinity ouabain binding site and low-dose positive inotropic effect in rat myocardium

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The mechanism of the positive inotropic effect of digitalis glycosides remains unclear. One theory suggests a causal relationship between the binding to and consequent inhibition of (Na⁺+K⁺) ATPase (the sodium pump) by digitalis and an increased myocardial contractile force¹⁻⁶. By this mechanism, the increased force of contraction would occur secondary to an elevation of intracellular sodium concentration which then causes an increased intracellular concentration of calcium via a sodium-calcium exchange mechanism4-6. Another theory proposes that the binding of digitalis to (Na⁺ + K⁺)ATPase causes an increase in a sarcolemmal calcium pool⁶⁻⁹, suggesting that the inotropic effect could be due to a causal relationship between the formation of the digitalis-(Na++K+)ATPase receptor complex and increased myocardial calcium availability and utilization, exclusive of an inhibition of the sodium pump⁷ We now report that two distinct positive inotropic sites for ouabain exist in rat ventricular strips. The higher-affinity response (ED₅₀ = 0.5μ M) correlates with an apparent high-affinity site which can be detected by ³H-ouabain binding to intact rat ventricular myocytes. These higher-affinity sites do not correlate with concentrations (IC₅₀) of ouabain necessary to inhibit (Na++K+)ATPase activity of sarcolemma preparations prepared from rat ventricles, suggesting that in the rat ventricle the high-affinity site for the inotropic effect of ouabain may not be related to inhibition of (Na++K+)ATPase. The low-affinity site is, however, related to inhibition of this enzyme.

Figure 1 shows a typical cumulative dose-response curve of ouabain on isometric contractile force in rat right ventricular strips. In a series of identical experiments, a 'low concentration

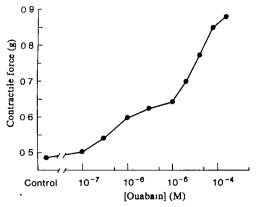


Fig. 1 Typical biphasic effect of ouabain (in mol l⁻¹) on isometric contractile force (in g) in rat ventricular myocardial strips. Right ventricular strips were prepared from adult rats as previously described¹¹ and vertically mounted in a water-jacketed muscle bath containing (in mM) 137 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.05 MgSO₄, 0.4 KH₂PO₄, 12 NaHCO₃ and 10 glucose. This Tyrode solution was aerated with 95% O₂-5% CO₂ and maintained at a pH of 7.0 at 35 °C. The strips were allowed to equilibrate for 60 min while electrically paced at 1 Hz (via punctate platinum electrodes) with a stimulus duration of 2 ms and voltage at 20% above threshold (6–8 V) During this equilibration period, the preload was constantly adjusted to 1 g. A single cumulative concentration-response curve for ouabain was obtained for each ventricular strip. For the experiment shown, the apparent ED₂₅s were estimated to be 0.5 and 19 μM.

effect' occurred between 0.1 and 10 µM ouabain, with an increase in contractile force of $34 \pm 5\%$ (n = 25) above control, while a 'high concentration effect', with a maximal increase of $84 \pm 9\%$ (n = 25) above control contractile force, occurred with 80-160 µM ouabain. Although the increase in contractility was undoubtedly produced by a complex series of events, the obvious biphasic nature of the ouabain dose-response curve (Fig. 1) is consistent with the existence of two separate glycoside receptor sites. We have therefore estimated two ED50 values from the experimental data. The high-affinity site had an ED₅₀ of $0.53\pm0.08 \,\mu\text{M}$ (assuming that the inflection point in the curve at 10⁻⁵ M represents the maximum effect of this site) and the low-affinity site had an ED₅₀ of $19\pm2\,\mu\text{M}$. Neither β adrenoreceptor blockade (20 µM sotalol) nor pretreatment of the rats with reserpine had any effect on the biphasic nature of the curve, the maximal increases in contractile force or the apparent ED₅₀ values for the low or high concentration effects, but the low-dose effect could be removed by prolonged washing. Erdmann et al.10 recently reported that rat ventricular strips had a single ouabain inotropic receptor with an ED₅₀ of 0.3 μM. This value is lower than found by us¹¹ and may be due to the fact that Erdmann et al. 10 were unable fully to elicit the high concentration effect, because in their preparations toxicity occurred at ouabain concentrations >30 µM.

In the same experimental conditions used to study rat ventricular strips, we obtained only a monophasic concentration-response curve for rat left atria (ED₅₀ = $45 \pm 9 \,\mu$ M), which is consistent with earlier reports of the positive inotropic effects of digitalis glycosides in rat myocardium¹¹. Similarly, only monophasic response curves were obtained for cat, rabbit and guinea pig ventricular papillary muscles or left atria. Thus, two inotropic receptor sites for ouabain seem to exist in rat ventricle that are perhaps less easily detectable in rat atria or myocardium of the other species.

Although most studies of ${}^{3}H$ -ouabain binding to various membrane preparations and purified $(Na^{+}+K^{+})ATP$ ases reveal a single class of ouabain binding site 12 , several workers have recently obtained results consistent with the existence of two populations of such sites ${}^{11,13-17}$. In an effort to detect two receptor sites of different affinities, we elected to examine ${}^{3}H$ -ouabain binding to isolated myocytes 18,19 instead of to membrane preparations, for two main reasons. First, it is likely that cell membrane preparations from ventricular myocardium contain $(Na^{+}+K^{+})ATP$ ase(s) from more than one cell type, including fibroblasts and postganglionic sympathetic nerves, which probably have different affinities for ouabain, particularly in

the rat¹⁷. Second, the preparative procedure for cardiac sarcolemma can potentially alter the configuration of all membranes and thus artificially create more than one apparent population of receptors.

The method of Akera and Cheng²⁰ was used to examine 3 H-ouabain binding to isolated rat ventricular myocytes. A $K_{
m D}$ value can be calculated from data obtained in a displacement assay (Fig. 2) by subtracting the concentration of the labelled ouabain (a) from the concentration of unlabelled ouabain ($C_{0.5}$) which causes a 50% displacement of the labelled ligand. Using concentrations of labelled ouabain of $0.1 \,\mu\text{M}$ (n=3) and $0.2 \,\mu\text{M}$ (n = 3), we obtained from probit plots $C_{0.5}$ values of $0.225 \pm 0.066 \,\mu\text{M}$ and $0.322 \pm 0.028 \,\mu\text{M}$, respectively, to give an average $K_D \pm s.d.$ of $0.124 \pm 0.090 \mu M$. Akera and Cheng²⁰ emphasize that this calculation of K_D applies only to a single class of site and that the binding reaction must be at equilibrium. Although equilibrium is difficult to obtain in ouabain-sensitive species, which have slow dissociation rates^{5,21,22}, equilibrium binding of ³H-ouabain to rat cardiac (Na⁺+K⁺)ATPase can be achieved rapidly due to a high dissociation rate^{5,21}. We found 30 min sufficient for equilibrium binding in our studies (data not shown). Because of large variability in the data obtained at the higher concentrations of unlabelled ouabain (where labelled ligand binding is low) we could not determine whether or not there was a second, lower-affinity site for ouabain binding. Such a low-affinity site clearly exists, as the IC₅₀ for inhibition of our rat cardiac sarcolemma preparation is 50 µM, similar to previously reported values for rat heart (Na⁺+K⁺)-ATPase^{11,21-25}. This low-affinity site for enzyme inhibition agrees well with that for positive inotropy (Fig. 1). Thus, our results are not consistent with the conclusion of Erdmann et al.10 that there is a complete dissociation of the positive inotropic effect of ouabain from the low-affinity site.

Studies reported by Godfraind and Ghysel-Burton²⁶⁻²⁸ on ouabain uptake, tissue electrolyte content and contractile force in guinea pig atria, and experiments reviewed by Noble²⁹ on

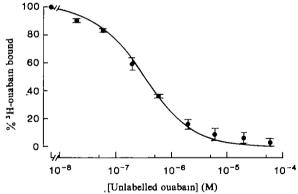


Fig. 2 ³H-ouabain binding to intact ventricular myocytes, isolated from adult rat myocardium as previously described^{18,19} The curve shows the effect of increasing amounts of unlabelled ouabain on the binding of 0.2 μM ³H-ouabain (100% binding) to myocytes in the presence of Krebs-Ringer bicarbonate buffer containing (in mM) 118.5 NaCl, 2.6 KCl, 0.5 CaCl₂, 1.18 MgSO₄, 1.18 KH₂PO₄, 14.5 NaHCO₃, 11.1 glucose and 2.0% (w/v) bovine serum albumin (Miles fraction V); the final incubation volume was 2 ml To each assay tube (5 ml Teflon beakers) 200 μl of ³H-ouabain (1 μM, 17 Ci mmol⁻¹ in aqueous solution) were added with appropriate amounts of unlabelled ouabain (stock solutions prepared in Krebs-Ringer bicarbonate buffer). After preincubation for 15 min in a shaking water bath (37 °C) aerated with 95% O2 and 5% CO2 in a closed hood, the binding reaction was initiated by the addition of 0.5 ml of cells ($\sim 4 \times 10^4$ cells). The reaction was terminated after 30 min by transferring the incubation beakers to an ice bath, followed by rapid filtration on Whatman GF/C glass fibre filters. The beakers were rinsed with 10 ml cold KCl (100 mM) and this rinse was used to wash the filters (filtration and wash time was ~5 s) The filters were placed in vials and incubated for at least 10 h in scintillation fluid before determination of radioactivity. External standards were prepared using ³H-ouabain and nonspecific binding was defined as the bound radioactivity not displaced by 5.5×10^{-3} M outbain, which was $\sim 30\%$ of the total binding in the absence of any added unlabelled ouabain. The curve shown is predicted from the equation, $\%^3$ H-ouabain bound = $100/(1+C/C_{0.5})$ where C = concentration of unlabelled outbain and $C_{0.5} = K_{\rm D} + a$, with $a = 0.2 \,\mu{\rm M}$ and $K_{\rm D} = 0.125 \,\mu{\rm M}$. Each point represents the mean (±s.e.m.) of three separate assays with a concentration of 0 2 µM 3H-ouabain.

potassium-sensitive reversal potentials, current-voltage relations and twitch tension in sheep Purkinje fibres, have suggested the presence of two ouabain binding sites of high and low affinity, related to Na+,K+-pump stimulation and inhibition, respectively. Low doses of ouabain (~10⁻⁸ M) can often produce a small but reversible negative inotropic effect, which may be transient^{29,30}. Our results, obviously consistent with the presence of two ouabain binding sites, show a reproducible positive inotropic effect of ouabain at levels corresponding to binding of the glycoside to a high-affinity site. This positive inotropy cannot be causally dependent on an overall decrease in sodium gradient if the direct or indirect effects of low doses of ouabain are to produce a net stimulation of the Na+,K+pump²⁹

The striking discrepancy between the apparent IC₅₀ value for ouabain inhibition of rat heart (Na⁺+K⁺)ATPase and the $K_{\rm D}$ of the higher-affinity ouabain binding site reported here, suggests that there may be no relationship between Na+,K+pump inhibition and the low concentration inotropic effect in rat ventricle. However, the high-affinity ouabain binding site may represent only a small fraction of the total number of $(Na^+ + K^+)$ ATPase molecules in rat ventricle, the majority being of the lower-affinity type. In this case, inhibition at the higheraffinity site by low concentrations of ouabain may produce a limited increase in internal sodium (and secondarily of calcium), because the pump still has sufficient 'gain' to keep internal sodium relatively low. At higher glycoside concentrations, binding to the low-affinity site reduces pump gain and internal sodium will rise rapidly. The exact shape of the force-binding curve will depend on the relationship between internal sodium and force, which is not simple³¹, but a biphasic response would not be surprising. This hypothesis is difficult to test experimentally, as we cannot detect a change in rat cardiac (Na++K+)ATPase activity at ouabain concentrations below 1 μM. Alternatively, the higher-affinity site may exist as a catalytically inactive conformation or isozyme of (Na++K+)-ATPase. In this case, high-affinity binding of ouabain in vivo would not result in pump inhibition, but perhaps induce a conformational change in the enzyme (and enzyme-associated membrane lipids) which could increase calcium binding to sarcolemma^{7,9,15}.

Thus, ouabain binding to the higher-affinity $(Na^+ + K^+)$ -ATPase in rat ventricular sarcolemma could result in increased calcium mobilization and availability to the myofibrils, producing the low-concentration positive inotropic effect in isolated rat ventricular muscle. These effects could occur independently of Na⁺,K⁺-pump inhibition. With increasing concentrations of ouabain, binding to the lower-affinity site would occur, resulting in Na⁺,K⁺-pump inhibition, enhanced Na⁺-Ca²⁺ exchange and a more pronounced positive inotropic effect. While there appears to be a clear distinction between the two sites as well as between the two inotropic phases in rat ventricular myocardium, these differences in myocardium of ouabain-sensitive species may be small and thus difficult to detect if, in fact, two populations of ouabain sites actually exist.

This work was supported by USPHS grants PO1 HL 22619 and F32 HL 05802, the American Heart Association, Southwestern Ohio Chapter, the British Heart Foundation and the MRC.

Received 17 August 1981, accepted 21 January 1982

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Nanomolar concentrations of extracellular ATP activate membrane Ca channels in snail neurones

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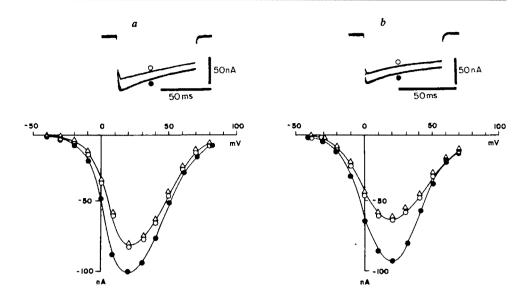
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Intracellular ATP is known to affect the electrical properties of membranes indirectly by acting intracellularly as a substrate for ATPases such as (Na++K+)ATPase, Ca2+-ATPase or adenylate cyclase. More recently, extracellular ATP has been shown to act on effector cells innervated by autonomic nerves and a purinergic receptor hypothesis has been proposed for this effect^{1,2}. Extracellular ATP also acts on the slow inward current. In of cardiac muscle membranes 3,4 where the effect may be coupled to a change in intracellular metabolism. These observations raise the possibility that extracellular ATP (ATP.) might alter the Ca current of excitable membranes. The Ca current is of major interest because it acts as the trigger for many cellular functions such as secretion, contraction, motility and luminescence. To test our idea, we examined the effects of ATP. on a well studied Ca current, that of neuronal membranes. As we report here, ATP, in nanomolar to micromolar concentrations produced substantial increases of I_{Ca} , and the non-hydrolysable congener of ATP, adenylylimidodiphosphate (AMP-PNP) had an even greater effect. By contrast, intracellular perfusion of ATP in nanomolar quantities had no effects on I_{Ca} , but with 10⁻⁵-10⁻³ MATP small increases were observed. Intracellular perfusion with AMP-PNP at these doses had no effect on I_{Ca} . We conclude that ATP, enhances membrane Ca channel activity by an as yet undetermined mechanism.

The experiments were performed on identifiable neurones from the suboesophageal ganglia of Helix aspersa, usually F1, F2 and E1, E2 according to Kerkut et al.5. The nerve cell bodies were isolated by dissection and aspirated into a suction pipette for internal perfusion and voltage clamp⁶⁻⁸. A separate intracellular micropipette provided the membrane potential signal for the clamp circuit. K currents were suppressed by tetraethylammonium (TEA) 50 mM extracellularly, 4-aminopyrine (4-AP) 5 mM extracellularly, TEA 10 mM intracellularly, and substitution of K ions by Cs ions intra- and extracellularly. Na currents were suppressed by Tris substitution for Na ions extracellularly and Na ions were absent from the intracellular solution. Intracellular Ca activity was buffered to 10⁻⁸ M by 0.1-1.0 mM EGTA. In these conditions two currents are present over the potential range of -50 to +50 mV-a linear, non-voltage-dependent, non-time-dependent leakage current and I_{Ca} (ref. 8). Leakage and linear components of capacitative currents were eliminated by adding to a pair of voltage-clamp

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Fig. 1 Effects of extracellular ATP (a) and AMP-PNP (b) on I_{Ca} (current was corrected for linear components of leakage capacitance by the subtraction method described in text). The compositions of perfusion solutions (in mM) were: internal: Csaspartate, 135; TEA-OH, 10; Tris-base, (pH 7.3); external: Tris-Cl, 35; CsCl, 5; TEA-Cl, 50; CaCl2, 10; MgCl2, 15; 4-AP, 5; glucose, 5.5, Tris-base, HEPES, 20 (pH 7.4). All chemicals were added to the control solutions immediately before use. a, Upper panel shows the effect of ATP (10^{-6} M) on I_{Ca} elicited by a 70 ms pulse to +20 mV from a holding potential of -50 mV before (O) and 15-20 min after (●) the addition of ATP. Bottom panel shows voltage-current relationships of peak Ca current control (O), 15-20 min after ATP (10⁻⁶ M) (•)



and 60 min after washout (\triangle). b, Upper panel shows the effect of AMP-PNP (10^{-8} M) on I_{Ca} with the same pulse protocol as in a before (\bigcirc) and 15 min after (\bigcirc) the addition of AMP-PNP. Bottom panel shows voltage-current relationships of peak Ca current control (\bigcirc), 15-20 min after AMP-PNP (10^{-8} M) (\bigcirc) and 60 min after washout (\triangle).

steps from the holding potential, $V_{\rm H}$, of $-50\,{\rm mV}$ the current responses that were equal in amplitude but opposite in sign. At potentials more positive than $+50\,{\rm mV}$, a time-dependent, nonspecific outward current also appears but these potentials were rarely used in the present experiments and when they were used, nonspecific leakage current was corrected for by subtracting the current that remained after blockage of $I_{\rm Ca}$ by Co substitution for Ca.

The effects of extracellular ATP and AMP-PNP were observed after control currents had been steady for 30-60 min (see Fig. 1). ATP in concentrations of 5×10^{-9} to 10^{-8} M produced an immediate increase in I_{Ca} which continued until a new steady-state level was attained within 5-10 min. Maximum effects were observed at about 10^{-4} M ATP and were similar for Tris, Na and Li salts of ATP, the effects being reversible. AMP-PNP produced the same effect as ATP (Fig. 1b). The increases in I_{Ca} amplitude occurred without any apparent changes in the kinetics of activation, and although inactivation occurred at a slightly faster rate for the largest currents, this may be related to Ca-current-dependent inactivation^{8,9}. There were no changes in leakage currents. The increase in Ca currents was not voltage dependent (Fig. 1a, lower panel), as the curves of peak current versus voltage before and after ATP were simply scaled versions of each other. This rules out a surface charge effect due to ATP adsorption which would also be unlikely at such low concentrations. As the peak Ca currents are little affected by inactivation, which is at least 10 times slower than activation, and as there is no steady-state inactivation of I_{Ca} at the holding potential of -50 mV used in these experiments, the ATP_o effects must be due mainly to an increase in the channel steady-state activation process. This could result from an increase either in the number of conducting units or in the average unit conductance of channels already present.

Figure 2 shows the dose–response relationship for ATP_o and AMP-PNP_o actions on peak I_{Ca} . The respective average maximal increases were $125\pm16\%$ and $130\pm16\%$ (mean \pm s.d.) with n=6 for ATP_o and n=7 for AMP-PNP_o. The K_0 , is 10^{-6} M for ATP and 3×10^{-9} M for AMP-PNP. At higher concentrations $(10^{-3}$ M) the response falls from its maximum value but we have not further investigated the response at these unphysiological concentrations.

One possible explanation of how ATP_o activates Ca channels is that ATP acts as a substrate for an outward-facing membrane ATPase. However, this would require hydrolysis of the molecule, which is ruled out by the effects of the non-hydrolysable congener, AMP-PNP. Another possibility is an outward-facing

adenylate cyclase, but extracellular cyclic AMP had no effect on I_{Ca} . Alternatively, ATP may permeate these particular neuronal membranes and act as a substrate for adenylate cyclase located in the membrane interior. Investigation of this possibility by perfusing ATP and AMP-PNP intracellularly revealed that ATP produced small increases of I_{Ca} , but required concentrations of ≥10⁻⁵ M to achieve the effect, and that AMP-PNP was ineffective even at 10⁻² M. The effects appeared within 10 min and were steady by 15 min. Hence, the present results are not due to ATP_o acting as a substrate for a membrane system that activates Ca channels. They may be due to a specific reaction between ATP_o and the membrane, as suggested by the purinergic hypothesis, or a mechanism similar to that proposed for the β -adrenergic receptor reaction $^{10-12}$ might exist. In this case the ATP-receptor complex would activate adenylate cyclase to increase cyclic AMP and thus I_{Ca} . Extracellular cyclic AMP had no effect on I_{Ca} but intracellular perfusion at concentrations of 10⁻⁵ M produced a small increase. This hypothesis would require that the complex amplify adenylate cyclase activity because ATP applied intracellularly in micromolar quantities had much smaller effects than ATP or AMP-PNP applied extracellularly in nanomolar amounts. Extracellular application of ATP might then be enhanced by prior intracellular perfusion with ATP. We tested this proposal using millimolar concentrations of ATP, and found that further increases of I_{Ca} occurred in response to ATPo. However, the effects seemed to be simply additive and the results were inconclusive. We therefore tried

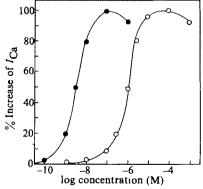


Fig. 2 Dose-response curves for the effects of extracellular ATP (\bigcirc) and AMP-PNP (\blacksquare) on I_{Ca} (elicited by a 70 ms long pulse to +20 mV from holding potential of -50 mV). Each point represents the mean of six measurements for ATP and seven for AMP-PNP obtained from different neurones (curves were drawn by eye).

the opposite experiment, to deplete the cell of ATP, and observe the effect of ATPo. To do this we perfused internally with oligomycin, 10⁻⁶ M (Sigma), or ruthenium red, 5×10⁻⁴ M (Sigma). Although the response to extracellular ATP was reduced in these experiments, the Ca currents in the presence of the metabolic inhibitors were also declining, thus preventing any firm conclusion regarding cyclic AMP as a second messenger.

We studied the potency of phosphorylated adenosine and guanosine compounds and found the sequence to be AMP-PNP » ATP > ADP, with AMP and adenosine having no effect. We also found that GTP (Sigma) was about as effective as ADP and that GMP-PNP was as effective as AMP-PNP. Furthermore, the putative purinergic antagonists, apamin (Sigma) 5× 10⁻¹⁰ M, quinidine (Sigma) 10⁻⁶ M, propranolol (Sigma) 10⁻⁶ and phentolamine (Sigma) 10⁻⁷ M, had no effect on the response to ATPo. Thus, from the above results the mechanism whereby ATPo activates Ca channels remains uncertain.

This work was supported in part by DHHS. NIH grants NS-11453 and HL-25145.

Received 28 September 1981; accepted 13 January 1982.

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Human melanoma-associated antigen p97 is structurally and functionally related to transferrin

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p97 is a 97,000-molecular weight (MW) cell-surface glycoprotein, which is present in most human melanomas but in only trace amounts in normal tissues1-4. We describe here the purification of p97 by affinity chromatography with monoclonal antibody, followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and determination of the N-terminal amino acid sequence using a new, highly sensitive protein sequencer⁵. The sequence was found to be homologous to the N-terminal sequences of transferrin and lactotransferrin. This structural homology was confirmed by the observation that antiserum to denatured p97 cross-reacted with denatured transferrin and lactotransferrin. We have also demonstrated that p97 is functionally related to transferrin and lactotransferrin in that it binds iron. This is one of the first reports of the amino acid sequence of a human tumour-associated cell-surface antigen and one of the few cases in which insight has been obtained into

p97 was purified by affinity chromatography. Monoclonal IgG2a antibody specific for p97 was added to a detergent lysate of melanoma cells, and the mixture was passed through a protein A-Sepharose CL-4B column. The antibody and p97 were eluted from the adsorbent at pH 5. The proteins were reduced with 10 mM dithiothreitol (DTT) and alkylated with 50 mM iodoacetamide, and p97 was separated from the IgG heavy and

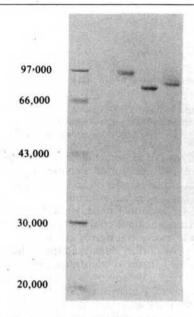


Fig. 1 Purification of p97. SK-MEL 28 melanoma cells (10 g) were suspended in 40 ml 20 mM Tris-HCl buffer pH 8.0, containing 100 mM NaCl, 1 mM EDTA and 0.5% Nonidet-P40 (TNEN buffer) supplemented with 1 mM phenylmethylsulphonyl fluoride (PMSF), and the lysate was centrifuged at 300,000g at 4 °C for 1 h. Antibody $96.5 (5 \mu g ml^{-1})^4$ was added, and the lysate was passed through a 1 ml column of protein A-Sepharose CL-4B at 4 °C. The column was washed, then eluted with 2 ml of 100 mM citrate buffer pH 5, containing 0.5% Nonidet-P40. The proteins were precipitated with 60% methanol containing 0.6% acetic acid at -20°C and electrophoresed on a SDS-6% polyacrylamide gel as described elsewhere 14, except that 5 mM sodium mercaptoacetate was added to the sample buffer. The gel was stained with Coomassie blue, and p97 was eluted by overnight incubation at 20 °C in 0.2 M Tris-acetate buffer pH 8.0, containing 0.1% DTT and 1% SDS, followed by electrophoretic elution in 50 mM Tris-acetate buffer pH 7.8, containing 0.5 mM sodium mercaptoacetate and 0.1% SDS, and then in 50 mM ammonium bicarbonate containing 0.1% SDS. The figure shows a stained SDS-10% polyacrylamide gel of: a, molecular weight markers phosphorylase b (97,000), serum albumin (66,000), ovalbumin (43,000), carbonic anhydrase (30,000) and soybean trypsin inhibitor (20,000); b, purified p97; c, transferrin; d, lactotransferrin.

light chains by preparative SDS-PAGE. Approximately 50 µg of p97 were obtained from 10 g of cells. The purified protein electrophoresed as a single, narrow band on an analytical SDSpolyacrylamide gel (Fig. 1). It appeared to be slightly smaller than phosphorylase b (MW 97,000) and significantly larger than human transferrin and lactotransferrin (MW ~80,000). The immunological specificity of the purification procedure was established using control IgG2a antibodies. As p97 comprised < 0.1% of the protein in the initial cell lysate, the two-step procedure resulted in a > 1,000-fold purification.

Amino acid sequence analysis⁵ of purified p97 yielded a sequence of 12 residues at the N-terminus (see Fig. 2). A computer search of the Newat amino acid sequence library6 revealed that this sequence is homologous to the N-terminal sequence of transferrin7, seven of the 12 residues being identical (Fig. 2). We consider that this homology is significant for two reasons: first, the two least common amino acid residues, Trp and Cys, are among those conserved, and second, six of the seven conserved residues are also conserved at the N-terminus of lactotransferrin8 (Fig. 2). On the basis of the limited amount of sequence data available, p97 appears to be as closely related to transferrin and lactotransferrin (~50% identity) as these proteins are to one another. For comparison, haemoglobin α and β chains have 44% identity⁶. We conclude that p97, transferrin and lactotransferrin have evolved from a common

Independent evidence for the structural relationship between p97 and transferrin and lactotransferrin was obtained by

p 9 7	Gly Met Glu	Val Arg Trp Cys Ala Thr Ser Asp ? G	ilu
Transferrin Val Pro	Asp Lys Thr	Val Arg Trp Cys Ala Val Ser Glu His G	lu l
Lactotransferrin Gly Arg Arg	Arg Arg Ser	Val Gin Trp'Cys Ala Val Ser Gin Pro G	alu

Fig. 2 N-terminal amino acid sequences of p97, transferrin and lactotransferrin. The N-termini are shown at the left. In the p97 sequence, the Gly and Ser residues are tentative, the Cys residue was detected as S-amidomethylcysteine phenylthiohydantoin, and the twelfth residue was not detected. Conserved amino acid residues are boxed.

serological analysis. An antiserum obtained by immunizing mice with denatured p97 recognized denatured p97 and cross-reacted with denatured transferrin and lactotransferrin (Fig. 3). As a negative control, serum albumin was not recognized by the antiserum. On the other hand, we did not observe cross-reactions when mouse monoclonal antibodies to native p97 and rabbit antisera to native transferrin and lactotransferrin were tested. We conclude that denatured p97 shares antigenic determinants with denatured transferrin and lactotransferrin, and that these antigenic determinants presumably are conserved amino acid sequences. Antiserum to denatured p97 may prove useful in searching for additional members of the transferrin family.

Transferrin and lactotransferrin bind iron with high affinity. To determine whether p97 also binds iron, we incubated melanoma cells with ⁵⁹Fe, lysed them with non-ionic detergent, added monoclonal antibody to the lysate and passed the mixture through a protein A-Sepharose column. When antibodies specific for p97 were used, the column retained ~4% of the ⁵⁹Fe, showing that a significant proportion of the iron taken up by the cells was bound to p97. In control experiments using no antibody and four antibodies not specific for p97, including two antibodies specific for other melanoma cell-surface proteins, <0.05% of the ⁵⁹Fe was retained (Fig. 4). We conclude that p97 at the surface of viable melanoma cells can bind iron.

On the basis of the similar molecular weights of p97 and the transferrin receptor on SDS-PAGE after reduction, it has been suggested that two proteins are identical⁹; however, our results do not support this. First, the tissue distribution of p97 differs

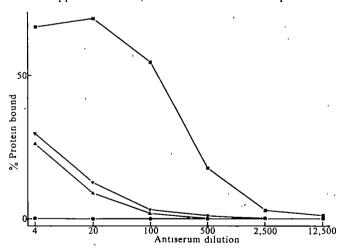


Fig. 3 Serological cross-reaction of p97 with transferrin and lactotransferrin. An antiserum to denatured p97 was obtained by immunizing BALB/c mice with p97 that had been reduced, alkylated and purified by SDS-PAGE. The mice were given ~5 μg antigen emulsified in 0.5 ml complete Freund's adjuvant intraperitoneally, were boosted 4 and 6 weeks later with antigen in incomplete adjuvant, and were bled 2 weeks after the last immunization. Proteins radioiodinated as described previously to a specific radioactivity of 10⁸ c.p.m. μg⁻¹, and purified by SDS-PAGE without previous alkylation (4,000 c.p m in 15 μl TNEN buffer) were incubated with 5 μl antiserum at 0 °C for 2 h. Formalin-fixed Staphylococcus aureus (10 mg) were added in a volume of 1 ml and after 5 min at 0 °C were pelleted and washed with 1 ml TNEN buffer. Normal mouse serum tested as a negative control bound <0.5% of each protein. ■, p97, ▲, transferrin; ●, serum albumin.

from that of the transferrin receptor^{2,9-11}. Second, on SDS-PAGE without previous reduction, p97 migrates slightly faster than the reduced protein⁴; in these conditions the transferrin receptor migrates as a dimer with a MW of ~200,000 (refs 9, 12). Third, monoclonal antibody OKT9, which is specific for the transferrin receptor⁹, did not immunoprecipitate p97 from radioiodinated melanoma cells, and antibodies specific for p97 did not immunoprecipitate the transferrin receptor from radioiodinated melanoma or leukaemia cells. Sequential immunoprecipitation experiments confirmed that the molecules immunoprecipitated by these antibodies were distinct. Finally, p97 did not bind to a transferrin-Sepharose column (data not shown). Clearly, p97 and the transferrin receptor described⁹⁻¹¹

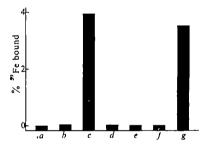


Fig. 4 Binding of 59 Fe by p97 Confluent SK-MEL 28 cells were washed three times with 25 ml phosphate-buffered saline (PBS) pH 7.2, and incubated at 37 °C for 1 h with 10 ml PBS containing 2 mM NaHCO₃, 1 mM sodium citrate and 10^7 c.p.m. 59 FeCl₃. A cell lysate, prepared as described in Fig. 1 legend, contained 4×10^4 c.p.m. 59 Fe. Monoclonal IgG2a antibody ($20 \, \mu g \, \text{ml}^{-1}$) was added to the lysate, and the mixture was passed through a 0.2 ml protein A-Sepharose CL-4B column, which was washed with 2 ml TNEN buffer. Results are expressed as % ⁵⁹Fe retained by each column: a, no antibody; b, antibody 103.1, specific for a cell-surface glycoprotein common to most human cells; c, antibody 96.5, specific for p97; d, antibody 10.9, specific for a mouse immunoglobulin allotype; e, antibody W6/32, specific for HLA-A, B, C; g, antibody 118.1, specific for p97.

are not identical, although amino acid sequence studies of the receptor should elucidate whether these molecules have any structural homology.

Our data show that p97 is structurally related to transferrin and lactotransferrin, and like these p97 binds iron. However, unlike transferrin and lactotransferrin, which are present in serum and milk (and other secretions) respectively, p97 is synthesized by melanoma cells and appears to be an integral membrane protein⁴. The presence of p97 at the surface of certain cell types suggests that its function may be translocation of iron.

Although p97 and the transferrin receptor are structurally distinct, their proposed functions are similar. Recent evidence confirms that the transferrin receptor recognized by monoclonal antibodies⁹⁻¹¹ does indeed mediate cellular uptake of transferrin-bound iron, as a monoclonal antibody specific for the receptor blocks uptake of transferrin and iron by leukaemia cells¹³. Our proposed function for p97 in iron translocation, however, has not been evaluated by functional studies. Given that normal adult tissues contain only trace amounts of p97, their iron uptake is more likely to be mediated by the transferrin receptor than by p97. The restricted tissue distribution of p97 (large amounts are present only in melanomas, nevi and fetal intestine)

implies a more specialized role for this protein, possibly related to particular aspects of iron metabolism in these tissues.

We thank Cynthia Green and Lynn DeOgny for technical assistance. This work was supported by NIH grants CA 27841, CA 25558, CA 14135, CA 19149 and GM 0695, grant IM 241 from the American Cancer Society and NSF grant PCM 80-

Received 28 October 1981; accepted 5 January 1982

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Ribosomal RNA transcription in vitro is species specific

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Eukaryotic cells possess three distinct nuclear DNA-dependent RNA polymerases which are responsible for transcription of different sets of genes (for review see refs 1, 2). Recently, cell-free transcription systems have been developed which faithfully initiate transcription of isolated genes by the corresponding RNA polymerase in the presence of crude cellular extracts. These cellular extracts supply additional components required for specific transcription³⁻⁵. Successful *in vitro* systems for transcription of RNA polymerase II or III genes were developed using either heterologous or homologous components⁷⁻¹¹. In contrast, an analogous cell-free system for the RNA polymerase I transcription unit from mouse has been shown to be active only with homologous extracts from mouse cells⁶. Data presented here show that in vitro transcription of ribosomal DNA isolated from mouse, human and a protozoan requires completely homologous components. None of the three active cell-free systems is capable of correct initiation on the nonhomologous templates. Further, supplementation of mouse extracts with purified protozoan RNA polymerase I failed to result in specific transcription of the protozoan rDNA, suggesting that the species specificity of pre-ribosomal RNA synthesis resides, in part, in the transcription factors.

It had been shown previously that specific transcription of rDNA from mouse requires transcription factors from mouse cells; neither human KB cell extracts nor frog oocyte or egg extracts could substitute for extracts from mouse cells⁶. One suggested explanation of this result was that species-specific factors might be involved in the expression of RNA polymerase I genes. We have investigated this apparent species specificity by assaying several different cloned ribosomal RNA genes for their ability to be selectively and correctly transcribed in the mouse extract system. Cloned ribosomal DNAs from Physarum polycephalum, Drosophila melanogaster and Xenopus laevis were used alone or in combination with mouse rDNA as templates in the cell-free transcription system derived from cultured Ehrlich ascites cells. The site of transcription initiation for each of these rDNA genes is known and thus the length of specific in vitro

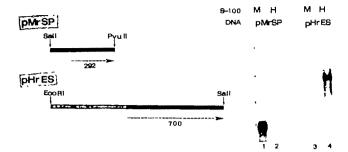


Fig. 1 In vitro transcription of rDNAs from mouse and man by homologous and heterologous cell-free transcription systems. One microgramme of cloned mouse rDNA truncated with PvuII (pMrSP/PvuII, lanes 1, 2) or human rDNA truncated with SalI (pHrES/SalI, lanes 3, 4) was incubated for 1 h at 30 °C in a 50-µl reaction mixture containing 10 mM HEPES (pH 7.9), 75 mM KCl, 5 mM MgCl₂, 0 05 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, 10 mM creatine phosphate, 600 μ M each of ATP, CTP and UTP, 50 μ M GTP, 3 μ Cı of [α - 32 P]GTP (specific activity 10–25 Cı mmol $^{-1}$) and 25 µl of S-100 extract S-100 extracts were prepared as described by Weil ecal from cultured Ehrlich ascites tumour cells (EA) or from HeLs cells (HeLa) Synthesis was terminated by addition of 50 µl or 100 mM sodium acetate, 0 5% SDS and 25 µg Eschenchia coli tRNA RNAs were extracted with phenol and chloroform, precipitated with ethanol and separated by electrophoresis on a 4% polyacrylamide gel as described by Weil et al.⁴. Autoradiograms of the gel are shown Lane 1 pMrSP/PvuII DNA+ Ehrlich ascites S-100 extract. Lane 2: pMrSP/PvuII DNA+HeLa S-100 extract Lane 3. pHrES/SalI DNA+Ehrlich ascites S-100 extract. Lane 4. pHrES/SalI DNA+HeLa S-100 extract

run-off transcripts can be predicted from restriction analyses and sequence data¹²⁻¹⁵. None of the heterologous rDNAs yielded specific transcripts (data not shown). The inability of these DNAs to support selective transcription was not due to the presence of inhibitory components in the template preparations because they did not reduce correct transcription of the homologous mouse template in mixing experiments. Thus, although the mouse S-100 extract is capable of directing correct initiation of transcription on mouse ribosomal DNA, it seems incapable of recognizing the promoter for the ribosomal RNA transcription unit from other eukaryotic species.

The above experiments did not rule out the possibility that this apparent species specificity reflects a unique inability for promoter recognition by the mouse transcription system; extracts from other cell types might have a broader capacity to initiate correctly pre-ribosomal RNA chains. To test this, two systems derived from HeLa cells and Acanthamoeba, respectively, have been established in addition to the mouse system. In analogy to the mouse system, the human and protozoan S-100 extracts were prepared from logarithmically growing cells and were able specifically to transcribe human rDNA (pHrES) and rDNA from Acanthamoeba castellanii (pAr4), respectively (unpublished results). In the experiment shown in Fig. 1, the human and the mouse transcription system was used both with homologous and heterologous rDNA templates and in different combinations of the DNAs and the extracts. The mouse rDNA

Table 1 Specific transcription of rDNA from mouse (pMrSP) and man (pHrES) by homologous and heterologous S-100 extracts

rDNA used as		Specific
template	Source of extract	transcript*
Mouse	Ehrlich ascites (EA)	Murine
Man	EA	None
Mouse	HeLa	None
Man	HeLa	Human
Mouse	EA+HeLa	Murine
Man	EA+HeLa	Human
Mouse + man	EA	Murine
Mouse + man	HeLa	Human
Mouse + man	EA+HeLa	Murine + human

^{*}The murine transcript is a 292-base long run-off RNA from pMrSP/PvuII. The human transcript from pHrES/SalI is ~700 bases long.

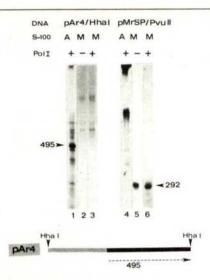


Fig. 2 In vitro transcription of rDNAs from Acanthamoeba and mouse in the presence of homologous and heterologous S-100 extracts and RNA polymerase I, respectively. A 915-base pair DNA fragment from the Acanthamoeba rDNA clone pAr4 (pAr4-Hhal) or a PvulI digest of the mouse rDNA clone pMrSP (pMrSP/PvulI) were used as templates and the transcripts analysed as described in Fig. 1 legend. S-100 extracts were obtained either from Ehrlich ascites tumour cells (M) or from log-phase A. castellanii cells (A). Reaction mixtures were supplemented (+) or not supplemented (-) with 0.015 U of Acanthamoeba RNA polymerase I purified through the heparin-Sepharose step as described elsewhere¹⁷. One unit of RNA polymerase will catalyse the incorporation of 1 nmol of UMP into RNA in 10 min in the conditions described 17. Lane 1: pAr4-HdaI DNA+A S-100 extract + RNA polymerase I. Lane 2: pAr4-HhaI DNA + MS-100 extract RNA polymerase I. Lane 3: pAr4-HhaI DNA+M S-100 extract+RNA polymerase I. Lane 4: pMrSP/PvulI DNA+A S-100 extract+RNA polymerase I. Lane 5: pMrSP/PvulI DNA+M S-100 extract-RNA polymerase I. Lane 6: pMrSP/PvuII DNA+M S-100 extract+RNA polymerase I.

clone pMrSP, when digested with PvuII at a site 292 base pairs downstream from the initiating nucleotide, yields a run-off RNA 292 bases long (ref. 15 and Fig. 1, lane 1). The human clone pHrES yields a transcript ~700 bases long after truncation with SalI (E.R. et al., manuscript in preparation, and Fig. 1, lane 4). However, when the mouse DNA (pMrSP/PvuII) is used as template in the human extract system, only low levels of nonspecific RNAs are produced (Fig. 1, lane 2). The same holds true if the human template (pHrES/SalI) is combined with the mouse S-100 components (Fig. 1, lane 3).

Mixing experiments presented in Table 1 revealed a pronounced template selectivity by the transcription factors for RNA polymerases present in the crude extracts. If both human and mouse rDNA were present in the assay, the source of S-100 determines which template is transcribed. Addition of equal amounts of extracts from human and mouse cells to a mixture of mouse and human templates results in the production of both the 292-base mouse RNA and the 700-base human run-off transcript. These results indicate that the cell-free transcription systems consisting of homologous RNA polymerase I and transcription factor(s) supplied by the S-100 extracts are only able to initiate correctly at homologous promoter sequences.

To verify this conclusion and to discover whether this apparent species specificity resides in the RNA polymerase I or the transcription factors, a third transcription system derived from A. castellanii was tested. This system yields a 495-base run-off RNA that coincides with the 5' end of 39S pre-rRNA when a HhaI-generated fragment of the Acanthamoeba ribosomal RNA repeat unit16 is transcribed in the presence of S-100 extracts from Acanthamoeba (Fig. 2, lane 1). RNA polymerase I purified from this species¹⁷ is incapable of selective transcription of this DNA in the absence of Acanthamoeba S-100 extract (Iida and M.R.P., unpublished results). Also, mouse S-100 extract yields no specific transcript with Acanthamoeba pAr4-HhaI DNA (Fig. 2, lane 2). When purified Acanthamoeba RNA polymerase I is added to mouse S-100 and used to transcribe pAr4-HhaI DNA, an increase in the 915-base 'end-to-end'

transcript but no selective transcription of pre-ribosomal RNA is observed (Fig. 2, lane 3). Supplementation of the mouse S-100 with the protozoan RNA polymerase I also does not stimulate transcription of mouse rDNA (Fig. 2, lane 6); only nonspecific RNAs increase slightly in amount. Thus, supplementation of the nonhomologous transcription factor-template mixture with homologous RNA polymerase I does not result in the synthesis of specific RNA, suggesting that the role of the transcription factor involves recognition of the promoter sequence or the polymerase or of both.

The data presented here strongly suggest that the recognition of rDNA occurs in a species-specific manner. This is in contrast to RNA polymerase II and III transcription systems, which show little or no species specificity. Comparison of nucleotide sequences in front of RNA polymerase II or within RNA polymerase III genes have revealed a remarkable sequence conservation in defined positions necessary for accurate transcription initiation 18-22. In contrast, a comparison of the sequence in the region of transcription initiation of different ribosomal genes has revealed no obvious shared consensus sequence among several eukaryotic rDNAs tested 12-15,23-25 This lack of a homologous sequence in the rRNA genes is in accord with our finding that species-specific factors are involved in the transcription of rDNA by RNA polymerase I. A change in the single promoter sequence of this transcription unit, if complemented by a similar modification of the polymerase I (or the transcription factor) DNA binding site, could be a reasonably frequent genetic variation. In addition, because the ribosomal RNA transcription unit is repeated many times per cell, genetic experimentation with altered promoter sequences may be a relatively facile (and seldom lethal) event. Apart from the constraints which might arise as a result of the need to keep the repeated genes co-evolving, genetic drift of the rRNA promoter may be comparatively rapid. Conversely, RNA polymerase II * deals with thousands of different single copy gene promoters and, because there is probably no direct mechanism to keep them co-evolving, they must remain true to the prototype TATA-box sequence to remain active.

We thank Drs R. Braun, P. Wellauer and R. Reeder for the gifts of rDNAs from Physarum polycephalum, Drosophila melanogaster and Xenopus laevis, respectively, and Dr G. Wilson for the human clone pHrES. This work was supported by grants from the Deutsche Forschungsgemeinschaft (I.G.), the NIH (GM 22580, GM 26059) and by a Senior International Fellowship (F06-TW00524) of the Fogarty International Center (NIH) to M.R.P.

Received 20 November 1981; accepted 6 January 1982.

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Lipidic particles and cubic phase lipids

RECENTLY Sen et al. have observed1 regular arrays of inverted micelles in galactolipids and correctly depicted their molecular organization. However, the authors did not differentiate the observed inverted micelles from lipidic particles as seen in phosphatidylethanolamine and cardiolipin2-5. The inverted micelles observed by Sen et al. are, in fact, units of an inverted cubic phase first proposed by Luzzati and Reiss-Husson⁶. The inverted cubic structure has recently been observed in glucolipids by proton NMR7 and in monoglycerides by X-ray diffraction8.9. The dimensions reported by Sen et al. in galactolipids (8-9 nm) are not too different from those measured by X-ray diffraction in sunflower monoglyceride8 (5.8 nm) and in synthetic monoglycerides (4.5 nm). Unfortunately, Sen et al. did not recognize the cubic structure seen in their electron micrographs, and they did not correlate their findings with existing X-ray diffraction and NMR data of similar lipids. Instead, they compared

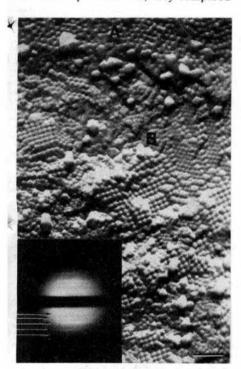


Fig. 1 Freeze-fracture electron micrograph of dilinoleoylphosphatidylethanolamine and egg phosphatidylcholine (mol ratio 85:15) dispersion. The sample was heated to 40 °C and cooled to 24 °C before being freeze-quenched in liquid propane without cryoprotectant, using the quick-freeze sandwich method13. Conical pits (lipidic particles) in area A are randomly distributed but become regularly distributed in area B, and spherical units (shadowed opposite to the pits) of a cubic structure are seen between these pits. Small-angle X-ray diffraction was taken from the same dispersion. The five reflections indicated by arrows are identified in the text. Scale bar, 100 nm.

those spherical inverted micelles with the conical 'lipid particles' seen in phospholipids2-

It is important to note that the 'particles' shown in ref. 1 are of a different nature from the 'lipidic particles' discussed previously^{2-5,10-12}. We illustrate this by showing (Fig. 1) a rapidly quenched freeze-fracture (without cryoprotectant) micrograph of mixed egg phosphatidylcholine and dilinoleoylphosphatidylethanolamine (mol ratio 15:85) at 24 °C after being heated to 40 °C. The conical lipidic particles observed in area A are the usual type of bilayer attachment observed in phosphatidylethanolamine-containing lipid mixtures. In area B, the cusps are arranged in a square array, trapping pockets of water between bilayers, thereby forming a complementary array of spheres.

These water-containing spheres, as units of an inverted cubic phase, would be truly inverted micelles were it not for their possible interconnecting water channels7,8. They should not be confused with the lipidic particles, which are the inter-bilayer attachment sites^{5,10-12} surrounding these inverted micelles. The size of the spheres is 17.8 nm in our micrograph, corresponding to our X-ray diffraction data, which show the (200), (220), (222), (422) and (440) diffractions at 8.7, 6.2, 5.1, 3.6 and 3.2 nm of a cubic structure (Fig. 1, inset). Our model for the involvement of lipidic particles in the bilayer-inverted cubic transition will be presented elsewhere.

In conclusion, we have shown that the inverted cubic phase can be observed by electron microscopy, and that this structure occurs not only in glycolipids but also in fully hydrated phospholipids. The units of the inverted cubic phase are inverted micelles, which are distinguishable in shape and size from the so-called lipidic particles or cusps.

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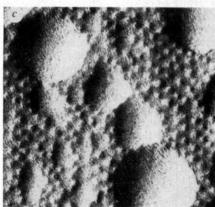
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SEN ET AL. REPLY-Hui and Boni are correct in pointing out that the term 'lipidic particle' has been used in the literature to describe a number of different structures. Unlike these authors, however, we believe these structures to be closely related.







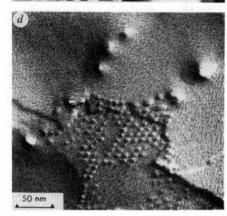


Fig. 2 Electron micrographs of freeze-fracture replicas prepared from sonicated dispersions of mono- and digalactosyldiacylglycerols mixed in a molar ratio of 2:1. a, Sample containing 58.4% ethylene glycol; b, sample containing no cryoprotectant thermally quenched from 50 °C; c, 58.4% ethylene glycol sample; d, sample containing no cryoprotectant quenched from ~20 °C. Samples were prepared and replicated as described elsewhere5

We have been able to identify at least four distinct types of lipidic particle from electron micrographs of freeze-fracture replicas of aqueous dispersions of mixed galactolipids. Figure 2 provides a composite view of some of these. The particles shown in Fig. 2a and b are regular quasicrystalline arrangements induced by the addition of ethylene glycol and by heat, respectively. Figure 2c shows isolated pits formed from particles of the type seen in Fig. 2a. A two-dimensional array of particles located within a single fracture-face is shown in Fig. 2d together with a number of rather larger particles that resemble structures attributed by some workers to inter-bilayer attachment sites1

The quasi-crystalline structures, as we pointed out in our original communication⁵, seem to correspond to aggregates of inverted lipid micelles. Hui and Boni have suggested that such structures correspond to isotropic cubic phases of a type previously reported in X-ray diffraction studies of soaps and detergents⁶ monoglycerides^{7,8}. simple In our experience, a variety of quasi-crystalline structures characterized by different particle sizes and symmetries can be induced to form in mixed galactolipid dispersions. We are, consequently, less willing than Hui and Boni to commit ourselves to any definite symmetry assignments. We do not exclude the possibility that such structures are related to cubic phases similar to those referred to by these authors but, as we have emphasized elsewhere9, more evidence based on other techniques is required before any realistic attempt can be made to analyse the detailed structure of the aggregates we have reported.

Hui and Boni further suggest that the occurrence of cusps formed by groups of pits associated with inverted lipid micelles present in these aggregates are directly related to the structures attributed to inter-bilayer attachment sites 1-4. This resemblance is in our opinion purely fortuitous and merely reflects the fact that such aggregates contain a continuous three-dimensional hydrocarbon matrix. We believe that the various types of lipidic particles reported in the literature can be accounted for in terms of a series of intermediary states made up of inverted micelles at one extreme and bilayer arrangements in which the lipids forming these micelles have been re-incorporated into the bilayer phase at the other.

This reorganization, as detailed elsewhere 10 involves an appreciable expansion of the surface area of bilayer. In the initial stages, this leads to blebs of the type seen in the fracture-face shown in Fig. 2c, and then to a buckling of the fracture-face as shown in Fig. 2d. This process can account both for the formation of tubular-micelle structures and particle-strings of the type shown in Fig. 1 of our original report⁵ and the characteristic arrangements of ridges and particlestrings reported in other studies.

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Gene duplication and the birthday problem

EDLUND and Normark¹ have presented sequence and distribution data of tandem duplications of the ampC gene of Escherichia coli K-12, which suggest that recombination between unequal homologous sequences of the order of 12 base pairs (bp) in length may be a general mechanism for the generation of tandem duplications. In support of this conclusion they present calculations showing the probability of occurrence of homologies 10-14 bp long in a DNA sequence of given length. For example they state that on average two homologous sequences of any composition will be found within a random DNA sequence of 4 kilobases (kb)".

It is more realistic to calculate the length of DNA required such that the expected or average number of homologies is one. The number of homologies will be expected to be approximately Poisson distributed and the expected number of homologies, λ , will be given by

$$\lambda = n \frac{e^{-r/n}}{k!} \left(\frac{r}{n}\right)^k \tag{1}$$

where r is the length of the DNA segment, n is the number of possible different sequences of length m, equal to 4^{12} for a sequence 12 bp long, and k is the number of copies of a homology. Thus, on the average, one pair of homologous sequences 12 bp long will occur in a DNA sequence of length 5.8 kb rather than 4 kb as stated by Edlund and Normark. Allowing for the 1 kb sequence of the ampC gene, this value becomes 6.8 kb. The corrected values for Table 1 in ref. 1 may be obtained easily from equation (1).

Table 1 (ref. 1) does not make clear that the probabilities of pairs of homologous sequences are being calculated, rather than the total number of sequences which are homologous in a DNA segment of given length. Although the size of DNA required to obtain an average of four homologous pairs of length 12 bp is 11.6 kb (not allowing for the ampC gene), the length of DNA required to obtain an average of one sequence occurring four times is 580 kb! This point is significant as the opportunity for unequal recombination is much greater if multiple copies of a sequence are present than if several pairs of different sequences are present.

A further complication is that the calculation of the probability of occurrence of a sequence homology assumes that the DNA segment of length r contains r-m+1 (approximately r) independent sequences of length m. This is clearly incorrect as neighbouring sequences overlap and their probabilities of occurrence will depend on each other. If this dependency is ignored, then the frequency of sequence homologies will be overestimated.

These calculations do not alter Edlund and Normark's conclusion that unequal recombination between random homologies could be a mechanism for generating tandem duplications of the size they observe. This size, 9.8 kb, is still larger than the estimate of the minimum length of DNA required for a homology of length 12 bp to occur an average of once, even if this estimate is biased. However, the importance of such random homologies for generating tandem duplications significantly shorter in length may be questionable.

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- 1. Edlund, T. & Normark, S. Nature 292, 269-271 (1981). Feller, W. An Introduction to Probability Theory and its Applications 3rd edn (Wiley, New York, 1968).
- NORMARK REPLIES—Edlund and I recently suggested1 that unequal recombination between perfect homologies of the order of 10-14 bp may be a general mechanism for generating duplications in the size range of 10 kb. This suggestion was based on DNA sequencing of one duplication in the ampC region of the E. coli chromosome, on end point determinations of a number of duplications and on probability calculations of occurrence of homologies.

Our calculations that are questioned by Adams must be regarded as approximations. They were made in order to see whether or not our distribution of duplications could be explained by recombination betweeen short homologies of any base composition. Our calculations and Adams's do not give very different results. The main drawback with both calculations is that we do not know whether or not short homologies are distributed at random in *E. coli* DNA.

What is needed are computer scans for short homologies in continuous stretches of sequenced *E. coli* DNA. At present >5 kb has been sequenced in the *amp*C region of the *E. coli* chromosome (Yaurin and Grunchstrom, personal communication). This region is, however, still too short for such an analysis. Moreover, the recombination site for a number of independent duplications has to be DNA sequenced. Not until then will it be possible to test the calculations made by Adams and our group.

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The Red Sea line and Arabian-Nubian magmatism

HARRIS AND GASS¹ suggested that the Red Sea rift developed along the line of a late Proterozoic suture and that compositional differences in the upper mantle and/or lower crust on either side of the suture controlled magmatism within the Arabian-Nubian shields during the Phanerozoic. We now discuss both the existence of the proposed late Proterozoic suture and the nature and distribution of granitic magmatism in the region.

The hypothesis that the present Red Sea rift was controlled by a late Proterozoic suture is unjustified, because it can be demonstrated that there is structural and lithological continuity from the Nubian shield segment across the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the Archive children in the Red Sea rift zone to the R

rift zone to the Arabian shield segment. Figure 1 shows a reconstruction² of the Arabian-Nubian shield before Red Sea rifting. South of latitude 20°N (all localities identified by coordinates are on the Saudi Arabian side of Fig. 1) lineaments are oriented N-S, that is, angled at between 20° and 90° to the trace of the proto Red Sea fracture. There is good lithological correspondence between rocks assigned to the Mormora, Adola, Tambian and Tsaliet stratigraphical units in Ethiopia3 and rocks assigned to the Baish, Bahah, Hali and Jiddah units in Saudi Arabia⁴⁻⁶. Radiometric age data from Saudi Arabia indicate that this distinctive lithological belt was formed between >900 and ~600 Myr ago7.

Between latitudes 20° and 24° N, northeast structural trends predominate and the basement fabric is approximately normal to the proto Red Sea fracture. Major faults and distinctive folds can be traced from north-east Ethiopia and north-east Sudan into the west-central Arabian

shield, and lithological correlations can be made between stratigraphical units such as the Nafirdeib (>750 Myr), Asoteriba (~660 Myr), Awat and Homogar of Sudan⁸ and the Samran (>760 Myr) and Fatimah (~680 Myr) of Saudi Arabia⁹.

North of latitude 24°30′N the predominant trend in the basement is northwest) and in a few places, for example, between latitudes 26° and 27°N, the basement fabric is consistent with the trace of the proto Red Sea fracture. In this region the Farri (>900 Myr), Al Ays (725–800 Myr) and Hadiyah (~670 Myr) units of Saudi Arabia¹⁰ can be lithologically matched with the Abu Ziran, Dokhan and Hammamat units of south-east Egypt^{11,12}.

The strong north-west trending basement fabric persists north of 27° N within the corridor produced by the Najd strikeslip fault system. This system traverses the proto Red Sea fracture into the Egyptian segment of the Nubian shield. In this region the Zwam Nusfat and Thalbah-Hadiyah-Shammar units of north-west Saudi Arabia¹³ are possibly equivalent to the Abu Ziran, Dokhan-Hammamat units of the central Eastern desert of Egypt.

Whilst basement structures may have locally influenced the development of the Red Sea rift it is highly improbable that the rift was fundamentally controlled by a late Proterozoic suture. Such a cryptic suture would have to traverse at least three major structural provinces in which either north-west, north-east or north-south trending structures are dominant. Furthermore, the remarkable similarity between late Proterozoic stratigraphies of countries bordering the Red Sea is incontrovertible evidence that such a suture did not exist.

Harris and Gass propose that the late Precambrian calc-alkaline granites of the Arabian shield originated in a wet mantle above a subduction zone, whereas, the peralkaline granites resulted from partial melting of dry mantle after subduction had ceased. This hypothesis does not explain why (1) in the area east and southeast of Yanbu Al Bahr, near the central Red Sea coast in the Saudi Arabian shield, peralkaline granitic plutonism occurred some 50-100 Myr before a major episode of calc-alkaline granitic plutonism (D.B.S. and J. Aleinikoff, work in progress), and (2) in general, voluminous calc-alkaline and peralkaline granitic magmas were emplaced in the northern half of the Saudi Arabian shield contemporaneously either in the same pluton^{14,15} or in the same region^{1,10,14,16}.

Finally we discuss whether the absence of undersaturated Phanerozoic magmas in the Arabian peninsula is due to fundamental differences in the chemistry of the upper mantle underlying the Nubian and Arabian shields¹. Whilst there is an apparent paucity of undersaturated Phanerozoic igneous rocks in the Arabian peninsula it is premature to say they are

absent. The undated feldspathoid bearing Sawda syenite pluton in the Midyan area¹⁷ is considered to be a possible correlative of the Egyptian nepheline syenites, and

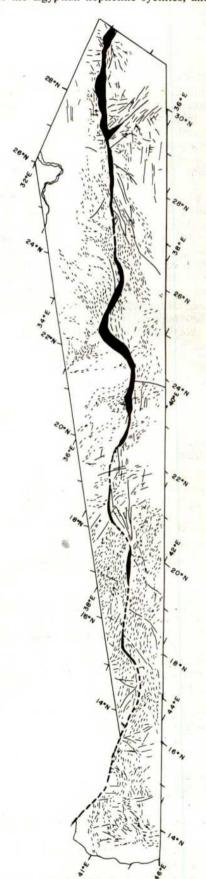


Fig. 1 Arabian-Nubian shield before Red Sea rifting.

peralkaline trachytes of unknown age puncture Palaeozoic rocks in the Tabuk area14. The chemical similarity of Cenozoic basalts in the Afar, Ethiopia¹⁸ and the Arabian peninsula 19 indicates that the mantle source region was not fundamentally different beneath the Arabian and Nubian shields.

As the Phanerozoic undersaturated alkaline magmatism in the northern Nubian shield has different character from that of the late Proterozoic Pan-African granitic magmatism, we see no reason to assume that this magmatism is an extension of the Precambrian episode. It would be more objective to say that the entire Arabian-Nubian shield experienced late Proterozoic calc-alkaline to peralkaline magmatism, and that a restricted province of predominantly undersaturated to peralkaline magmatism developed in the northern Nubian (and possibly Arabian) shield north region. Phanerozoic alkaline and peralkaline magmatism is found elsewhere in north Africa and that the Phanerozoic Nubian magmatism might be related to events in these regions.

We thank A. R. Drysdall for comments.

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HARRIS AND GASS REPLY—Jackson and Stoeser have not distinguished identification between the of Phanerozoic alkaline province in northeast Africa and our speculations on possible causes of this event and its apparent absence in Arabia.

Jackson and Stoeser identify an undated svenite and trachytes from north Arabia to counter our assertion that the Red Sea marks the eastern boundary of the Phanerozoic alkaline province. Future research may discover instances of pre-30 Myr Phanerozoic magmatism in the Arabian shield, but previous work has shown that the plethora of such intrusions in north-east Africa is not present in Arabia, Current geochronological studies on samples from both sides of the Red Sea substantiate the assertion that this igneous province represents the most significant geological contrast between the two sides of the Red Sea.

Following the identification of this igneous province we gave three reasons for its eastern margin being coincident with the Red Sea whilst emphasizing that more data are needed before we can be sure which of these is most relevant. Most of Jackson and Stoeser's comments are directed against our preference for the subduction zone model.

Many have commented on the apparent continuity of structures across the Red Sea, but with coastal plains covered with late Tertiary sediments and rotation of Arabia during the opening of the Red Sea the best that can be postulated is that there seems to be, in many places, structural continuity. The lithostratigraphical correlation between Arabia, Ethiopia, Sudan and Egypt has been shown to be unconvincing even within the southern Arabia shield¹, and correlation of illdefined and regionally unknown stratigraphical units in the northern Arabian shield is highly questionable. To extend this 'correlation' across the Red Sea with no geochronological or geochemical control is misleading. For example, results from geochronological studies from several of the Egyptian units mentioned by Jackson and Stoeser are totally at variance with their "lithological matching" of Arabian units. The conclusion that there is precise correlation through the Proterozoic across the Red Sea which precludes any relative movement between north-east Africa and Arabia during late Proterozoic times cannot be substantiated by available data. However, our prime purpose was to identify the Phanerozoic alkaline province in north-east Africa. We then argued that a post-500 Myr distinction in upper mantle geochemistry across the Red Sea line seemed likely. Finally we speculated that an eastward dipping subduction zone with a surface expression along the line of the Red Sea could explain the presence of alkaline magmatism in Africa and its seeming absence in Arabia. If future studies preclude such a suture, this will indicate that the Phanerozoic alkaline province in northeast Africa is the only crustal expression of the geochemical difference in the upper mantle across the Red Sea line until the opening of the Red Sea at 30 Myr.

Jackson and Stoeser imply that the peralkaline magmatism of Arabia is part of the calc-alkaline event, and requires no change in tectonic setting. Whilst the switch from calc-alkaline to peralkaline magmatism was diachronous, the appearance of metaluminous granitoids within peralkaline complexes does not support their view as such granitoids have 'within plate' trace element characteristics (such as high Nb) and are quite distinct from the widespread homogeneous calc-alkaline plutons². Such a marked change in high field-strength element abundance requires a variation in the volatile composition of the upper mantle which could be due to a change in tectonic setting. There are several granitic provinces, such as that of Mesozoic age in Nigeria³, which lie in a within plate tectonic setting and include peralkaline and metaluminous types both with characteristic high field-strength trace-element abundances. It seems realistic therefore to use the terms 'within plate' and 'subduction zone' based on diagnostic trace element abundances, rather than the now outdated calc-alkaliperalkali classification, to identify granitoid provinces.

We believe that the contrast in Phanerozoic magmatism in north-east Africa and Arabia is well-established and the points raised by Jackson and Stoeser do not challenge this. Its relationship with earlier subduction processes suggests that contrasting mantle geochemistry may prove to be the reason and, if so, the Red Sea line was defined in the mantle (and possibly in the crust) as early as 500 Myr.

We thank R. M. Shackleton, A. C. Ries and H. J. Duyverman for comments.

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in Nature. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should. wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

BOOK REVIEWS

Gerontology in adolescence

Alex Comfort

THE most promising omen for experimental gerontology is that it has emerged from the infancy-period of all-explaining general theories into that of specifics. The exercise is to understand and control age changes: these, in a complex organism (human beings would be the obvious beneficiaries - nobody needs long-lived mice), are bound to be multifarious. The first target is to ascertain which time-linked changes are lifespan determining, and the second to establish where, at the supragenetic level, these are coordinated. Coordinated they must be, in view of the high stability of specific lifespans and their divergence in related species.

The first two volumes of this report of a symposium convened by L'Institut de la Vie deal respectively with biology and with medicine and social science. (Volume 3, Behavioral Sciences and Conclusions, will be published later this year.) The first, gerontological, volume is an excellent introduction to the diversity of current research topics and the state-of-the-art in each. In contrast to older, one-process views of ageing, the growth stocks in current age studies are immunology, endocrinology, non-actuarial measures of the ageing process - essential, if we hope to look for interventions which modify it in organisms whose lifespans are of the same order as that of the investigator — and, over-arching these, the possibility that the peripheral changes are modulated by a neuroendocrine timekeeper. The odds are that there are some clocks running which are coordinated in this way, and others, possibly including the finitude of cellular clonal division (reviewed in this book by its discoverer Hayflick) which are autonomous.

Two excellent reviews of cerebral ageing, by Scheibel and Scheibel, and by Hachinski and Obrist, open the symposium. Immunology is reviewed by Walford, and hormones by Bierman, Adelman and Everitt — all distinguished investigators. What is lacking, though, is an integrativespeculative model. The discussion of the chances of lifespan modification, opened by the late George Sacher, is largely taken up with the potential of genetic engineering, to the exclusion of the other, and more accessible, programme store, the hypothalamus; and there is little or no consideration of the one authentic instance of age-retardation, that produced by calorie restriction in rodents, where the effect is almost certainly brain-mediated: much of the endocrinological evidence cited by Aging: A Challenge to Science and Society. Vol. 1 Biology, edited by D. Danon et al., pp.346, ISBN 0-19-261254-9; Vol. II Medicine and Social Sciences, edited by A.J.A. Gilmore, W.M. Beattie et al., pp.403, ISBN 0-19-261255-7. (Oxford University Press: 1981.) Vol. 1£25, \$59.50; Vol. II£30, \$59.50.

Adelman and Everitt points in this direction. The hypothalamus would certainly be an easier point of access than the genome, if we are undeterred by Sacher's pessimism over the social effects of success resulting in "demographic catastrophe".

That pessimism, in fact, assorts ill with the urgency of the problems raised by old age in the social and medical areas, the subject of Vol. II. Notably missing from both volumes is a full discussion of senile dementia, which, though a disease, not an "ageing effect", is now a socially ruinous epidemic and a subject of some of the most distinguished current research. There is, however, an excellent anatomical discussion by Terry in the biological section.

Perusal of the volumes leads to several conclusions - that gerontology has advanced enormously, from fringediscipline to major area of research; that in contrast to its teething years it now has more bricklayers than architects; and that possibly some of the researchers are lured by the prospects of success into long-term. genetic-engineering solutions, when they should be looking for answers nearer home which might have a more immediate clinical application. Clinical applications, not sci-fi projects aimed at radical lifespan change, are the most likely mode of entry for any results of research, and this entry cannot be stopped by social considerations, which may in any case be too alarmist.

Sacher, for example, concludes that

we should forgo our arguments about the desirability of life extension until we have done the kind of biologically-based actuarial and socioeconomic modelling that would give us at least a rough idea about the actuarial consequences of . . . life table alteration.

The hazard he envisages here — longer infirm and dependent life — seems biologically unlikely. More serious threats seem to follow from the fact that, while Vol.II of the symposium draws an excellent picture of the medical and social needs of the old under present actuarial conditions, society has so far proved resistant to providing these, and its response to longer health and longer working ability might be

equally negative and belated. A zeropopulation-growth society calls for longlived adults: having ceased to be like mice and rabbits, we might gain from becoming elephants. But, as Vol. II well illustrates, applied gerontology will undoubtedly be applied, as soon as it can be, to slowing or postponing disease changes - with the difference from current methods that the approach will probably be fundamental, and generate a whole field of ratedetermining medicine. It will, after all, probably be easier to postpone malignancy or immune failure than to reverse it once it has occurred. Examples already exist of cases in mice where diseases can be 'postponed out' of the lifespan.

This likelihood makes it extremely important to determine which of the deteriorative processes involved in the loss of homeostasis are self-timed, and which are under more general control and therefore potentially accessible *en bloc*—it is easier to change the policy of a company by tackling the chairman than by nobbling the sales representatives. On these grounds alone one may hope that an overall neurochemical "clock" will be identified.

It is, of course, possible, as Hayflick suggests, that relatively soon we may become able to address a higher level still, and make direct postnatal interventions in the genome. That would, probably, raise some policy questions, but those concerned with lifespan would be dwarfed by other and more general issues.

The most significant contributions to the second volume are Brocklehurst's discussion of training in geriatrics — a particularly urgent problem in the USA — and some short papers on geropsychiatry from the biochemical and genetic points of view. The demographic and social papers, apart from providing some menacing forecasts of what will result from continued neglect of the old, are more general, and a few, in the manner of social gerontology, verge on grannyology. Much of these may be summarized in a quotation from a notable letter to *The Sunday Times*:

I am a senior citizen [and] the following events may reasonably occur. (1) I shall have a gang of young thugs sent to paint my kitchen instead of going to prison (2) I shall have patients from the local mental hospital drafted to dig my garden (3) I may be forced to go to suitable entertainments, drink tea and wear a paper hat We . . are in a terrifying position: we are recipients. Hands off, please! I am in charge of my life.

That brief testimony should be inscribed

on the brows of the growing phalanx of "social gerontologists".

Although these proceedings are not the last word on age studies (the meeting was held four years ago and much has happened since that time) they make an excellent, authoritative and thoughtful review of the field. The papers by Hachinski and Obrist, by Scheibel and Scheibel, and by Ordy, on different aspects of brain ageing, merit reprinting where physicians will see them.

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Search for solutions

Barbara W. Low

Structural Studies on Molecules of Biological Interest: A Volume in Honour of Dorothy Hodgkin. Edited by G. Dodson, J.P. Glusker, and D. Sayre. Pp.610. ISBN 0-19-855362-5. (Oxford University Press: 1981.) £39, \$98.

THE arts and pleasures of praise, in writing, as in reading, may be direct or oblique. This *Festschrift*, written in honour of Dorothy Hodgkin, whose seventieth birthday it celebrates, abundantly manifests these several purposes with grace and rich diversity.

There are contributions from friends, colleagues and former students. Among these latter, Dennis Riley evokes the remarkable limitations of the early laboratory in the University Museum at Oxford (almost a gaslight era), and recalls their essential irrelevance. But he remembers, as we all remember, Dorothy Hodgkin's unique gift for proposing exciting problems without obvious means of solution and the exhilaration and triumph of their solution — learning to swim by being dropped in the deep end.

The section on insulin might be the transactions of a symposium, with all the original sense of convivial atmosphere and stimulating discourse. It provides a brief but fascinating account both of early studies and of those which have followed the determination of the insulin structure in Dorothy Hodgkin's laboratory. There is a continuity of theme in the contributions from different disciplines, for all are built around the concept of structure and function. No where is this more evident than in the account by Zhang You-Shang of the Shanghai Insulin Research Group. Zhang describes the investigation, using semi-synthetic methods, of insulin activity associated with modification of the C-terminal region of the B chain. The activity of p-Ala B23 deshexapeptide insulin is neatly accounted for in terms of the Ramachandran angles of B23 Gly

(normal residue) which lie, in the Ramachandran angle plot, within the permitted region for the p-configuration.

From the contribution of the Peking Insulin Research (Structure) Group, we learn that X-ray crystal structure and solution studies of the B chain C-terminal despenta- and desheptapeptide insulins are in progress, with their promise of a closer appreciation of structure/function relationships and the relationship between binding site and active site. Mercola and Wollmer provide an illuminating example of the enhanced understanding of solution phenomena which the high-resolution study of a protein may bring. This section also includes a nice discussion by Sakabe and colleagues of hydrogen bonding in the insulin structure at 1.2 Å resolution. These contributions abord in different ways the fundamental question of intrinsic molecular flexibility or rigidity, particularly at the binding site. I also enjoyed the discussion of Falkmer and Emdem on insulin evolution and Steiner's contribution on insulin precursors.

I have chosen to write of this section in the greatest detail because it represents not only the continuity of Dorothy Hodgkin's interests, but also a continuity of theme and focus. Crystallographers will especially enjoy the methods section, which includes a discussion by Bentley and Mason of the neutron diffraction study of monoclinic lysozyme and a contribution by Isaacs on fast-Fourier least-squares refinement. The more general section on protein structure includes an account by Harrison - "Approaches to the Structure and Function of Ferritin" — a history of crystal structure studies of the virus-like iron storage protein apoferritin. She concludes this provocative contribution with a reference to the isolation of a ferritin-like protein from Azotobacter vinelandii and from E. coli. Vainshtein also spans the range of common function by describing the structure of leghaemoglobin (from Lupinus nodule extracts) and comparing it with the functionally-related myoglobin.

There are also rewards elsewhere in the book. The contributions by Glusker on the vitamin B₁₂work and by Harding on the study of gramicidin S structure both provide a lively sense of the experimentalist tackling difficult problems by persistent frontal attack and by flanking approaches, reconsidering constantly as all evidence is re-evaluated. Thus difficulties may be approached almost by stealth — the coaxing of a problem to its solution.

There are many other stimulating and pleasing contributions. But to choose was here a regrettable necessity. The editors deserve our warmest congratulations and gratitude.

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Medicines worldwide

John Butterfield

Pharmaceuticals and Health Policy: International Perspectives on Provisions and Control of Medicines. Edited by R. Blum et al. Pp.272. ISBN 0-7099-0608-0. (Croom Helm/Holmes & Meier: 1981.) £17.95, \$37.50.

THE provision of medicines in the modern world is a complex process. It begins with the research stage; proceeds through toxicity testing and government-regulated clinical trials, drug licensing and registration; then passes into the distribution phase, which includes promotion by the manufacturers, dispensing by pharmacists and, in the case of certain medicines, prescription by the medical profession; finally it ends with consumption by patients. The beliefs and knowledge of the latter may critically affect patterns of actual drug usage in the home, a fact too often ignored by the professionals who direct the previous stages.

The authors of *Pharmaceuticals and Health Policy* collectively espouse the view that improved health care depends, in part, on the improved supply of medicines. To this end they present information about each of the key stages of the provision process and outline the various, sometimes conflicting, interests of the actors in the drama. Wisely, then, the editors have combined the skills of individuals from many disciplines — including medicine, psychology, administration, pharmacology, epidemiology, economics and sociology — and the result is an impressive amalgam of different viewpoints.

In tackling such a broad subject, the volume inevitably has its weaknesses. For example, despite the fact that readers are urged to identify themselves as "friends of research and discovery", little attempt is made to discuss the alternative forms of research management and funding which a community might adopt. Under what conditions is marked-funded, industrial research superior to state-funded, academic research? What acceptable or unacceptable "disbenefits" may be associated with each of these different modes of research support? Why have the international pharmaceutical houses been the source of so much of the pharmacological innovation of recent decades?

Similarly, despite a long essay on the individual use of medicines, no clear image emerges as to how the authors envisage consumers might be influenced to create more appropriate and productive behaviour in the field of drug usage. Personally, I would have preferred to see a more specific consideration of the needs and problems of that section of the population that conventional health education cannot reach, that is the poorer people—the people for whom education may be an anathema, who may be mentally less able

or emotionally less mature, but people who are the consumers likely to be at the greatest risk both of suffering drug-related mishaps and of not receiving suitable medication for treatable conditions. This is where health promotion must now be directed, and vigorously.

In addition, the volume does not adequately examine the consequences that national government policies have on the processes of medicine provision throughout the world. It is increasingly important that political interests are not exercised in areas such as drug registration, for example, where they could pass without the loud critical comment so often directed at academic, professional and commercial bodies.

But these cautions apart, the volume remains a worthwhile achievement. Lumbroso's essay on the introduction of new drugs and the problems faced by clinical trial organizers is of particular value. So too is Catherine Stenzl's concise chapter on the role of international organizations in medicine policy. Herxheimer and Lionel provide a sensible closing contribution on coherent policy formation, outlining the difficulties present and options open both nationally and internationally. It is with respect to the issues surrounding the latter area that the main messages of the book are to be drawn.

In the countries of the developed world, the home ground of pharmacological innovation, we have moved in the past few decades to a delicate balance between rival public, commercial, professional and governmental interests. "Structural maturity" may still not be fully established but the problems that are left are minor compared with those of 30, or even 20, years ago. In the rich world, at least, the contention that we are "reasonably well served by our drug providers" is now an entirely legitimate one.

Yet in the industrially less developed nations the situation is not so satisfactory. Not only are half or two-thirds of the people deprived of modern medication in more or less any form; but those who can obtain drugs do so in a local environment in which they may be open to many forms of harmful practice. These may be a result not only of ignorance, or lack of regulation, but also corruption — commercial, political and professional. In my view this is the central problem which drug providers throughout the world must face up to in the 1980s.

In his preface Dr T.A. Lambo, Deputy Director, WHO, says,

By my lights the reader [of this book] will be every government authority anywhere concerned with drug policy, anyone teaching clinical pharmacology, all concerned with cultural or social medicine, or medical economics, and most certainly my own

Erratum: the price of the paperback edition of *Principles of Neural Science* (reviewed in *Nature* 295, 474; 1982) is £19.95, not £25.50.

colleagues at every level who attend to international issues and collaboration in health affairs.

It certainly covers a very important subject - health policy. Those of us involved like to believe the quality of our work, of the decisions we make, the regulations we frame, the ethical motivation we achieve in the whole pharmaceutical process, are good indices of the quality of the society we seek to serve. The great trick from now on will be not simply to take care of the geese that lay the golden eggs - whether you regard those geese as the minds in the laboratory or the firms who develop and the clinicians who try the new medicines. It must also include ensuring that the eggs are safe for the children playing in the farmvard and - dare one use an old word? - that the charitable impulse of the medical and allied professions will prevail when the eggs get to the market places. All this is a complex network but there is no textbook for those involved. This slim volume will give newcomers valuable check lists based on the surveillance that the distinguished contributors have been maintaining.

Sir John Butterfield is Regius Professor of Physic at the University of Cambridge.

Great matters

Charles F. Kennel

Cosmic Plasma. By Hannes Alfvén. Pp.164. ISBN 90-277-1151-8. (Reidel:1981.) Dfl.75, \$39.50.

HANNES Alfvén's Cosmic Plasma is a highly individualistic essay about many topics central to contemporary space physics, astrophysics and cosmology. It deals with laboratory studies of cosmic plasma processes; the Earth's magnetosphere, ionosphere and aurora; Venus's magnetosphere; the solar wind and heliosphere; comets; the origin of the Solar System; interstellar clouds; cosmic rays; the question of antimatter in the Universe (to paraphrase Fermi: where is it?); quasi-stellar objects; and the expanding Universe. Alfvén's often rebelliously unconventional treatment of these topics leads him to conclude that plasma physics rules the behaviour of the Universe much more than is generally appreciated.

A basic premise of Cosmic Plasma is that our knowledge of laboratory and Solar System plasma physics must inform our understanding of more distant plasmas that we can neither manipulate nor probe in situ. To Alfvén, it is striking that spacecraft observe very different states of plasma separated by thin, stable current layers. We have learned, he says, that Solar System plasmas, and by implication all

NEW

GENETICS AND PROBABILITY IN ANIMAL BREEDING EXPERIMENTS

EARL L. GREEN

Director Emeritus of The Jackson Laboratory, Bar Harbor, Maine. Editor, Biology of the Laboratory Mouse, Second Edition.

Contents

Probability and Statistics; Segregation of Alleles; Assortment of Non-Alleles; Linkage, Recombination and Mapping; Mating Systems; Appendix 1 — Mean and Variance of a Binomial Distribution; Appendix 2 — Estimation of a Parameter by the Method of Maximum Likelihood; Appendix 3 — Extensions of the Method of Maximum Likelihood; Appendix 4 — Comparative Efficiency of Matings for Detecting and Measuring Linkage; Appendix 5 — the Fibonacci Sequence; Appendix 6 — Systems of Mating; Appendix 7 — Numbers of Mating; Appendix 8 — Nomenclature; Appendix 9 — Record Keeping; Appendix 10 — Mouseroom Layout and Procedures; Glossary of Signs; Literature Cited; Index.

This book is a primer and reference on probability, segregation, assortment, linkage and mating systems for biomedical scientists who breed and use genetically defined laboratory animals for research.

It is organised so as to be useful to 'novices' and 'experts' alike. The former will find an orderly development of the probability aspects of transmission tests, segregation analysis, allelism tests, independence tests, linkage analysis and mapping. They will see how to design genetic experiments to yield data which can be analysed and they will also find a clear exposition of the theoretical consequences of various breeding systems which will enable them to make rational choices as to the kinds of animals to use in their own research.

Experts will find that the most frequently used formulae needed for estimating various genetic parameters and their random sampling variances are assembled in tables or are clearly set forth in the text and that the extensive table of contents and detailed index will enable them to find the necessary formulae quickly.

LEVEL: Professional, graduate, undergraduate reference.

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SCIENTIFIC & MEDICAL



MACMILLAN PRESS cosmic plasmas, are cellular or filamentary in structure. From this stems his astonishing conjecture about antimatter. The symmetry between matter and antimatter in the laws of elementary particle physics suggests that the Universe ought to have been created with roughly equal amounts of each. Our Solar System is made of matter, and the lack of evidence for antimatter elsewhere may mean that any primordial antimatter was annihilated when it came into contact with matter, leaving us with a matter Universe. However, Alfvén proposes that cells of matter and antimatter co-exist throughout the Universe, that the cells are kept apart by plasma forces and that annihilation is drastically slowed because it takes place only in the thin layers between cells. Antimatter is difficult to detect except by the radiation coming from its annihilation, which is much less intense than it would be if matter and antimatter were evenly distributed. We do not even know for sure, claims Alfvén, whether our nearest neighbour, a Centauri, is a star or an antistar. Annihilation, when it does occur, could provide the energy for the most violent cosmic phenomena we observe. For example, the mysterious quasi-stellars might be the result of an occasional staranti-star collision in the nucleus of an otherwise normal galaxy - if indeed a galaxy made half of antimatter is normal. And annihilation could have provided the energy for the expansion of the Universe. By weaving such (controversial) arguments, Alfvén elevates plasma physics to a position which is central to all of astrophysics.

Alfvén's track record compels us to take Cosmic Plasma seriously. He did not win his Nobel Prize for thinking the way others do. Forty years ago, he helped to create the foundations of magnetohydrodynamics. Twenty years ago, when this work had become central to space physics, he was already insisting that the most important effects occurred in thin current-carrying filaments, or sheets that separated uninteresting regions of "passive" plasma. There one would find small regions of strong electric fields parallel to the magnetic field existing in complete violation of magnetohydrodynamic theory. These electric fields were what accelerated particles to high energies in the aurora. Today we know that auroral electrons are accelerated about 5,000km above the poles of the Earth by electrostatic structures that bear Alfvén's name for them — double layers. He now asserts that the cosmic rays we observe are generated by undetectable double layers far above the poles of the Sun. The absence of antiparticles in the cosmic rays therefore does not argue against antimatter elsewhere in the Universe.

Is all of the physics presented in Cosmic Plasma correct? Many readers will find something to disagree with, just as I believe that altogether too much can be

understood assuming that cosmic rays above I MeV energy come largely from the Galaxy to accept Alfvén's Solar System origin for them uncritically. Some readers may wish to turn to Eugene Parker's excellent monograph, Cosmical Magnetic Fields (Oxford University Press, 1979), for a systematic development of the magnetohydrodynamic point of view Alfvén so thoroughly rejects. Is there another great idea or two lurking in Cosmic Plasma? It is too soon to tell. In any case, might the theoretician's cardinal sin be, not to be wrong, but to be uninteresting?

For whom is Cosmic Plasma intended? Certainly not the beginning student. It has been said that cosmic plasma theory proceeds from the cartoon approximation to the back-of-envelope approximation, and thence to detailed calculation and synthetic understanding. Alfvén believes that even our cartoons are all wrong, and so his discussion is rich in fresh cartoons. envelope calculations - and his own synthetic understanding - but is seriously lacking in the systematic development students need. One must know the literature thoroughly to follow Alfvén. Although he speaks directly to today's professionals, who can read Cosmic Plasma without a glossary, he fears they won't listen. Maybe he has tomorrow's young researchers in mind.

Alfvén's future biographers will find expressed in Cosmic Plasma his intellectual and psychological position vis à vis the conduct of science. Historians of science may perceive an interesting parallel. Just as it was given to Newton's generation to comprehend how gravity rules the Solar System, so has it been given to ours to discover and understand Solar System plasmas. Like Newton, Alfvén could not resist the temptation to contemplate the cosmic significance of what he had learned. Perhaps Alfvén's Cosmic Plasma is similar in intent to Newton's Letters to Richard Bentley. And the sentiments Newton expressed in the famous scholium to the Principia are echoed in the final lines of Cosmic Plasma:

It seems that so far no phenomena have been discovered which necessarily call for any new laws. The basic properties of a plasma seem to be the same, from the laboratory to the Hubble distance.

Charles F. Kennel is a Professor of Physics at the University of California, Los Angeles, and a member of its Institute of Geophysics and Planetary Physics.

Ten years after Moon-walking

David W. Hughes

The Moon — Our Sister Planet. By Peter H. Cadogan. Pp.391. Hbk ISBN 0-521-23684-3; pbk ISBN 0-521-28152-0. (Cambridge University Press: 1981.) Hbk £27.50, \$59.95; pbk £12.50, \$24.95.

ON DECEMBER 19, 1972, Eugene A. Cernan and Harrison H. Schmitt left the valley of Taurus-Littrow near the coast of the great frozen basaltic "sea" of Serenitatis. No one has been back to the Moon since. Twelve men wandered 90 km over its surface, spent 80 hours outside their spacecraft and brought back 381 kg of rock and soil.

Ten years on we can sit back and read about what has been learnt. Cadogan wrote his doctoral thesis on the carbon chemistry of lunar samples and worked on lunar dating after that. So we have an author who was at the sharp end of lunar research, taking time to write a book which traces our progress in understanding Earth's sister planet. Cadogan has provided us with a good, general review of the subject; a book which delves into lunar science to some depth and yet assumes no previous knowledge of mathematics or geology. He is to be congratulated on a job well done.

The book is split into six main sections. The first discusses the Moon as seen from a distance, and traces the advances in lunar cartography and geology and also the famous impact versus volcanism debate over crater formation. The second

condenses the enormous amount of information that accrued from the spate of American and Russian landers. Moon rocks and minerals are dealt with in the third section, and the next discusses the lunar soil and the way in which this is affected by the bombardment of micrometeorites, erosion by the solar wind and radiation damage inflicted by cosmic rays. Section 5 looks at the Moon as a whole and studies its shape, gravity, atmosphere, magnetism and heat sources. The final section turns to the problem of lunar origin, age and orbital evolution.

Cadogan's enthusiasm for his subject bubbles from every page, but at the same time he has taken great care to explain difficult concepts. The standard of the figures is excellent and the choice of illustrations and their positioning in the text leave nothing to be desired. The book is worth buying for the figures alone. I have, however, a minor point of criticism. Cadogan's style of English is idiosyncratic to say the least. Why should I be so generous? The style is awful. Even after 200 pages it still shocks one's sensitivity. This is not to say that the book is unreadable - in fact, I enjoyed reading it very much - but still the ghost of the illiterate scientist walks through every paragraph.

David W. Hughes is a Lecturer in Astronomy and Physics at the University of Sheffield.

Earth Sciences

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Biological Sciences

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AT I OUT CEME ITS

Awards

The Rank Prize Funds have announced the following awards: for Opto-Electronics, one prize worth £30,000, goes to Professor Calvin F. Quate (Stanford University, California) for his contribution to medical. biological and physical research through the concept of the scanning acoustic microscope, which uses sound rather than light to form images; the second Opto-Electronics prize of £10,000 goes to Dr Charles Eliott (Royal Signals and Radar Establishment Malvern, UK) for devising a novel infra-red detector. The Rank Prize for Nutrition is £20,000 and goes to Dr Hamish Munro (US Department of Agriculture) for his research establishing the balance of nutrients required as the body grows older.

Arthur C. Clarke (University of Moratuwa, Sri Lanka) has been chosen to receive the eighth Marconi International Fellowship Award in recognition of his scientific achievement for the benefit of humanity in the field of communications science or technology.

The Dr H.P. Heineken Prize for 1982 has been awarded to **Professor C. Weissmann** (University of Zürich) for achievement in the field of biochemistry or bio-physics.

Dr Martin D. Kamen (University of California, San Diego) is the recipient of the 1982 ASBC-Merck Award which recognizes excellence in biochemical research.

Dr Efraim Racker (Cornell University) is the recipient of the 1982 Herbert A. Sober Award for contributions to techniques in biochemical research.

Dr C.W. Daeschner Jr (University of Texas at Galveston) has been presented with the 1981 Sidney S. Kaliski Award of Merit by the Texas Pediatric Society.

The Zoological Society of London has made the following awards for contributions to zoology in 1981: The Scientific Medal to Profesor M.P. Hassell (Imperial College of Science & Technology) and Dr J.R. Krebs (Edward Grey Institute of Field Ornithology, University of Oxford); The Frink Medal for British Zoologists to Sir Eric Smith; The Thomas Henry Huxley Award to Dr N.R. Franks (University of Leeds); The Stamford Raffles Award to Lt. -Col. A.M. Emmet; The Prince Philip Prize to Jonathan Edward Greenland (The Grammar School, Bristol).

The biennial Artois-Baillet Latour Health Prize of four million Belgian francs will be awarded in 1983 for an outstanding contribution to the knowledge, the aetiopathogenesis, the diagnosis and the treatment of cancerous diseases of blood and the lymphatic system both of child and adult. Nominations to: The Secretary General, National Fund for Scientific Research rue d'Egmont 5, B-1050 Brussels before July 1982.

The International Association of Gerontology has created the Sandoz Prize for Gerontological Research. The prize, worth 20,000 Swiss Francs, will be sponsored by Sandoz Ltd. Basel/Switzerland to encourage research in all areas of gerontology and geriatric medicine including biological, medical, psychological, social and other relevant aspects with special emphasis on multidisciplinary research programs. Applications in English before 30 September 1982 to Prof. M. Bergener. Secretary General, International Association of Gerontology, Rheinische Landesklinik, Wilhelm-Griesinger-Str. 23, D-5000 Cologne 91, FRG.

Appointments

Sir Richard Bayliss is joining the Medical Services Study Group of the Royal College of Physicians of London as an assistant director.

Robert J. Beals (Hall China Co.) is to be the 84th president of the American Ceramic Society.

Sir Ralph Verney, has become a trustee of the School of Water Sciences, High Wycombe, UK.

Meetings

3-6 April, Medical Consequences of War to the Countries of Europe, Cambridge (Beer Davies, 1 Melville Rd, Edgbaston, Birmingham, UK).

5-6 April, Atherosclerosis: Mechanisms and Approaches to Therapy, London (Dr N.E. Miller, Dept of Chemical Pathology, St Thomas's Hospital Medical School, London SE1, UK).

5-7 April, Control Systems Concepts and Approaches in Clinical Medicine, Sussex (The Institute of Measurement and Control, 20 Peel St, London W8, UK).

5-7 April, The Jubilee Chemical Engineering Symposium, London (J. Ellis, IChemE, George E. Davis Building, 165-171 Railway Terrace, Rugby, UK). 5-8 April, Royal Society of Health Annual

Congress, London (Royal Society of Health, 13 Grosvenor Place, London SW1, UK).

6-7 April, Results and Potential of TMR as Diagnostic and Research Method, Oxford (Dr P. Hanley, Oxford Research Systems, Ferry Hinskey Rd, Oxford, UK).

7 April, Analysis of Surfaces and Interfaces in Electronic Devices, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

14-15 April, Risk, Cost and Pollution, Oxford (Mr L. Evans, Harwell Education and Training Centre, Harwell Laboratory, Oxon, UK).

14-16 April, The Assessment of Major Hazards, Manchester (Mr D.J. Hewitt, 121 Princess St, Manchester, UK).

19-21 April, 3rd International Recycling Congress, Berlin (IRC, Prof, Dr K.J. Thomé-Kozmiensky, Rhumeweg 14, D-1000 Berlin 37, FRG).

19-21 April, Cell Behaviour, London (Prof. R. Bellairs, Dept of Anatomy and Embryology, University College London, Gower St. London WC1, UK).

20-22 April, Life Sciences — Market Openings, Sheffield (A. Fraser, Frost and Sullivan Ltd, 104-112 Marlebone Lane, London W1, UK).

21 April, **Biocides in the Oil Industry**, London (Miss I.A. McCann, Institute of Petroleum, 61 New Cavendish St, London W1, UK).

27-29 April, Esarda Specialist Meeting, The Netherlands (Mr R.J.S. Harry, ECN, Postbus 1, NL-1755 ZG Petten, The Netherlands).

19-30 April, Commission for Climatology and Applications of Meteorology, Washington DC (Research and Applications Dept, World Meteorological Organization, Geneva, Switzerland).

30 April-3 May, Upper Jurassic and Cretaceous of Normandy, Le Havre (Dr J. Hancock, Dept of Geology, King's College, London WC2, UK).

30 April, Electron Beam Damage, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

2-5 May, Ceramics: A Space Age Technology, Cincinnati (The American Ceramic Society, Inc. 65 Ceramic Drive, Columbus, Ohio 43214, USA).

4-7 May, Secretory Immune System, New York City (The New York Academy of Sciences, 2 East 63rd Street, New York, New York, 10021, USA).

4-7 May, Council of Biology Editors, Louisville (P.L. Altman, Council of Biology Editors, Inc., 9650 Rockvill Pike, Bethesda, Maryland 20814, USA).

11 May, Transient Annealing of Semiconductors, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

11-13 May, ESTEC Spacecraft Electromagnetic Compatibility Seminar, Noordwijk (Mr H. Bachmann, Postbus 299, 2200 AG Noordwijk, The Netherlands).

10-14 May, Oil Pollution Control Course, Ipswich (Miss 1. McCann, Institute of Petroleum, 61 New Cavendish St, London W1, UK).

11-14 May, Industrial Corrosion, Geneva (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

nature

18 March 1982

Haig and Mr Malone's successor

The State Department should think hard before appointing a successor to Mr James Malone: first, it should redefine the job.

To be appointed Assistant Secretary at the State Department with responsibility for science and technology is to be offered a poisoned chalice. Few of the recent incumbents of the post have made a success of the job. Even strong-minded people such as Dr Dixie Lee Ray (in her time a doughty environmentalist and chairman of the Atomic Energy Commission as it used to be) have failed to make a success of it. Now Mr James Malone, the conservative lawyer appointed by President Reagan a year ago, has gone (see page 187) in a cloud of speculation that his error (of omission) is that he has not succeeded in delivering the new non-Carter policy on nuclear proliferation that the President has been promising since well before his election. Although it is easy to understand how a person whose links with the nuclear industry were widely advertised to Congress at the time of the confirmation of his nomination for the post should afterwards have failed to persuade congressmen of his impartiality on proliferation issues, the chances are that Mr Malone's failure is that of a man asked to tackle an impossible job. Before Mr Malone's successor is appointed, the State Department should give some thought to his job description.

That science and technology should be represented in some tangible form in the hierarchy of the State Department now goes without saying. International relations are now too important, and too intimately linked with science and technology, to be left exclusively to diplomats. Mr Malone's continuing responsibility for representing the United States at the Law of the Sea Conference is an example of what is expected of incumbents of this post. Carrying a new non-proliferation policy through the labyrinth of the government (Congress included) is another. Although problems of arms control are formally the responsibility of the Arms Control and Disarmament Agency, no assistant secretary worth his salt could fail to have opinions on these questions (and would be counted a failure if he did not, especially when as now the head of that agency must seem to many in the State Department to be Secretary of State in waiting). But there are also important and teasing questions to be decided about technological relations between the United States and other states. Just now, Japan has people at the State Department as exercised as the Soviet Union; beating back the tide of imported electronic goods on the American market is as preoccupying as deciding what should be done about the pipeline for natural gas between the Soviet Union and Western Europe or about scientists visiting the United States from supposedly unfriendly states.

The essential difficulty of the appointment is that it requires its incumbent at once to execute important parts of the administration's policy (negotiating an agreement on the Law of the Sea that will better suit the present administration's book, for example) and at the same time to provide advice on questions that are inherently subtle and perplexing. To make things worse, the job requires its holder to be able to win friends in Congress without being able to offer domestic patronage, and yet to be able to survive in the tricky waters of diplomacy (which means more than merely remembering to say "Thank you, Mr Chairman" after even an angry intervention in a United Nations committee meeting). The underlying dilemma, for Mr Alexander Haig as well as for Mr Malone's successor, is that the executive and advisory functions of the job are not easily compatible but that the conventions of Washington suppose that no mere

assistant secretary can expect his opinions to carry weight unless he can also demonstrate that he has executive responsibility for some part of the administration's programme.

There are two common solutions to such a problem, one of which is to appoint to such an impossible job a person so eminent that he or she can neglect or delegate administrative responsibilities. The other is to support somebody with no particular expertise by a high-level advisory committee which, however infrequently it meets, includes people who can be noisy on the telephone. The more imaginative but in the long run by far the most effective solution is to seek the best of both worlds — an incumbent whose reputation will ensure that his advice is listened to, and an advisory committee that will if necessary amplify what he has to say and some mechanism for making sure that the politics (domestic and international) are attended to. The State Department, having seen successive holders of this office sink beneath the waves, must now grit its teeth for the time when science and technology must be taken seriously — really seriously.

The case of non-proliferation is a good touchstone for the appointment of Mr Malone's successor. President Carter impaled himself unnecessarily on a policy about the proliferation of nuclear weapons that seemed to non-nuclear powers a splendid illustration of the cryptic imperialism of the United States and to would-be American suppliers of nuclear equipment to be a restraint on their legitimate trade. The Reagan Administration is right to look for a better balance, one that will acknowledge that it is no more possible to outlaw the proliferation of nuclear weapons by an act of Congress than it is to repeal the second law of thermodynamics by executive order. Whether it will find such a solution will depend on its success in persuading Congress that looking for loopholes in the International Atomic Energy Agency's safeguards system is less important than finding ways of dissuading potential nuclear powers from making bombs. The trouble is that doing that is as much dependent on the oldfashioned skills of diplomacy as on what new technology has to offer. In short, the State Department should resolve to be less formal in its requirements of a policy on non-proliferation; it should appoint a successor to Mr Malone who will help it to be just that.

Uneconomic savings

The financial squeeze on British universities is to continue. Structural change is the only defence.

The British Government is nothing if not resolute in its determination to spend less on higher education. The annual forecast of public expenditure which accompanied last week's budget shows that total expenditure will continue to decline even after the academic year 1983-84 — that by which the 8.5 per cent reduction decreed at the end of 1980 will have been completed. Because all sectors of higher education are lumped together in the figures, it is not possible to tell how the continuing contraction will be shared between the universities and other kinds of institutions, the polytechnics for example. But one thing is certain — if the government's plans stick, student numbers will fall further until the proportion of young people in higher education is little more than 11 per cent. Especially when the demand for

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higher education is increasing as quickly as at present, and when the government has itself provided for an increase in the cost of what is called "non-advanced further education" (vocational courses of various kinds), it makes no sense at all that, for the first time in British history, access to universities and to higher education in general should be restricted by decree of the Department of Education and Science.

Not even the British Government can be content with this illiberal state of affairs. Going down in history (and into a general election, now not more than two years away) with such a reputation cannot be much of a comfort. But those who will be permanently damaged are not the politicians responsible for these developments but educational institutions, universities and polytechnics, and the students whom they are now prevented from teaching. This is why it is simply not good enough that those who might be thought to be responsible for the long-term interests of the universities should for practical purposes be sitting on their hands, doing nothing to bring about the structural changes in higher education on which survival must ultimately depend. The University Grants Committee, which is technically responsible for foisting student quotas on its dependants, shows very little sign of advocating the changes in the mechanisms of financial support for universities that will be necessary if flexibility is ultimately to be achieved. The Committee of Vice-Chancellors and Principals, united (and successful) in its efforts in the past six months to establish that only the government can meet the cost of firing academics from tenured posts, is constitutionally incapable of pressing for arrangements that would encourage diversity within the university system. And the polytechnics, the other important sector of British higher education, are uncomfortably squeezed financially between local and central government yet as firmly isolated as ever from the rest of higher education. They suffer from the awkward circumstance that their bills are paid by local authorities, which are then promptly reimbursed by the central government. After two years of argument, the central government has wrested back only a token of control.

The folly of these arrangements is already clear. The student quotas to which universities are being required to plan for the academic year beginning eighteen months from now will ensure that almost all universities become smaller and thus make less efficient use of their capital equipment. Some will become so much smaller that they will be forced to abandon teaching in many fields and thus cease to be universities except in name. The obvious solution (see *Nature* 18 February, p.541) is to devise a mechanism that will enable the total cost to the government of supporting universities and their students to be defined in advance, and limited, without restricting the capacity of universities to perform the social functions of educating young people for which they were originally created. Such a device would also provide universities with an incentive towards diversity; universities fearful of their capacity to compete for students with, say, Oxbridge would be faced with the prospect either of making themselves distinctive or going out of business. Universities least able to compete would also find themselves persuaded by objective pressures to teach more economically or even to form associations among themselves whose effect would be an economical division of labour. What would be wrong with such a state of affairs except the unwillingness of individual universities to acknowledge that while they are titularly equal. some are more equal than others?

The practical obstacle to such an arrangement, however, is not so much the unwillingness of universities to acknowledge what must be done as the division between the two halves of the binary system — the universities on the one hand and the polytechnics created as a kind of academic counterforce by the late Mr Anthony Crosland in 1966. Both halves are now under severe pressure but independently. When academics are fired or teaching departments closed in either half of the binary system, it is in principle possible to ensure that the weakest will be the first to go. But there is no conceivable means by which the performance of people or departments in one half of the system can be compared with that of their equivalents in the other half. As a result, there is

every likelihood that the decisions now being made by universities and polytechnics separately are inconsistent, even nonsensical. Few among British taxpayers (many of whom are academics) dispute the need that the government should contain its public expenditure, but it is just as important that the cash limits now being enforced for higher education should be accomplished in the most economical way — that which will yield the greatest benefit for whatever funds there are to spend. After a long period during which the government has had to fight the local authorities for a measure of central control of the polytechnics, it is understandable that it should lack the stomach for trying to set up some common framework within which both kinds of institutions could function. But nothing less than that will meet the present urgent need.

Turkish illiberality

The scientific community must make up its mind how to help members denied freedom.

The sad case of Dr Yeter Ögelman (see *Nature* 25 February, p.638 and this issue, p.231), the Turkish scientist who spent more than half of last year in a Turkish gaol, is still unresolved. At the resumed hearing of her case last week, Dr Ögelman was neither acquitted nor found guilty of the charge that her part in the organization of a women's rights campaign in the early 1970s was subversive of the new social order in Turkey. The result is that the charge will remain in abeyance, allowing the authorities to breathe new life into it whenever they choose. Meanwhile, Dr Ögelman remains dismissed from her post in the physics department of Cukorova University.

What, in these circumstances, should the scientific community do? The first temptation is to protest that it is thoroughly illiberal, and a serious infringement of what has come to be considered ordinary people's rights, that a person should be hounded and imprisoned as Dr Ögelman has been. The difficulty, unfortunately, is that the scientific community is not competent to raise such a complaint. For Dr Ögelman has been prosecuted not because she is a physicist but because of her part in an organization intended to secure rights for women in a society in which these have recently been denied. So there is no cause for the scientific community or its academies to stand up and complain at Dr Ögelman's treatment. Exactly the same principle applies to other states, of which there are now all too many, in which scientists and others must work under conditions in which personal liberty is unreasonably restricted. Other people's laws may be distasteful, but they are, after all, laws from which people cannot be exempted because they are scientists.

So is there nothing that can be done for Dr Ögelman, and for the growing band of scientists imposed upon by martial law in Poland (and for that matter Argentina)? Formal protests are inappropriate — which is not to say that individuals should not be free to support other organizations that are competent to complain - but practical help is both appropriate and likely to be welcome. The obvious and shining example of what can be accomplished is that of the 1930s, when many of those for whom life had become intolerable in central Europe were able to find jobs and continue their careers in the West. Now, the plight of those whose freedom is constrained is rarely as desperate as in the years before the Second World War, and there is also something in the view that the cause of personal liberty itself will be helped if those in trouble with the authorities stay in their own countries. But the provision of refuges overseas is not the only practical help that can be offered and that is likely to be welcome. People who have lost their jobs can be helped to remain active in scientific work even from a distance. Grant-making bodies and those who organize international conferences can usefully consider the interests of their colleagues who are deprived of freedom. Is it too much to ask that the scientific community, rightly standing aloof from the internal affairs of states elsewhere, should nevertheless more systematically consider how to help those of its members who are in trouble?

Overseas science shake-up in US

Malone goes, Law of Sea again in limbo

Washington

The US State Department is carrying out a shake-up of some of its top officials responsible for international scientific and technological affairs. Last week, the department confirmed that Mr James L. Buckley, a former Republican senator who is now Under-Secretary of State for Security Assistance, Science and Technology, will become a direct assistant to Secretary of State Alexander Haig.

Mr Buckley's move coincides with the news, also confirmed by the department, that Mr James Malone is being removed from his post as Assistant Secretary of State for Oceans and International Environmental and Scientific Affairs, although he will remain head of the US delegation to the Law of the Sea negotiations which reopened in New York last week.

No official reasons have been given for the reshuffle. In the case of Mr Malone, who was previously a lawyer in private practice and had been general counsel of the Arms Control and Disarmament Agency under the Nixon Administration, sources in Washington suggest that there had recently been criticism of some of his staff appointments. It is also felt that Mr Malone had lacked sufficient time to provide adequate attention to a range of controversial policy areas for which his post is responsible.

The moves are also part of a general reorganization of the State Department which has followed the promotion of Deputy Secretary of State William Clark to the President's National Security Advisor. One result is expected to be a more pragmatic and less ideological approach to decisions involving technology and foreign policy.

The contraction of Mr Malone's responsibilities seems, in part, to be related to his failure to secure congressional backing for the proposed relaxation of restrictions on the export of nuclear technology.

Mr Malone, whose nomination to the State Department was backed by members of the right wing of the Republican party, and who has contributed to reports published by the conservative Heritage Foundation, ran into strong opposition during his nomination hearings over his previous links with the nuclear industry.

At the time it was revealed that, as a private lawyer, he had represented the nuclear industries of both Taiwan and Japan in negotiations with the United States over access to nuclear technology. He promised to distance himself from any policy decisions involving former clients, but his links with the industry raised questions about how far his efforts to reformulate the US position on nuclear non-proliferation reflected the views of the industry rather than the foreign policy interests of the nation as a whole.

Whether or not the charge was a fair one, it has limited the effectiveness with which Mr Malone has been able to argue the Administration's position. "Once his nomination had been approved, he did not help matters by producing position papers that clearly looked like industry documents," an aide to Senator John Glenn, one of the sharpest critics of Mr Reagan's efforts to boost the exports of the US nuclear industry, said last week.

Although no announcement has been

made about Mr Malone's successor, it is widely expected that several of his responsibilities for nuclear export policy will be transferred to Mr Richard Kennedy, Under-Secretary of State for Management as well as ambassador to the International Atomic Energy Authority.

Mr Kennedy was a member of the Nuclear Regulatory Commission between 1976 and 1980. Before that he served for five years on the staff of the National Security Council where he worked directly under Dr Henry Kissinger, and has therefore had wide experience of both nuclear and foreign policy.

Mr Malone's reassignment could also have an impact on the outcome of the Law of the Sea negotiations, where the United States is at the centre of two separate conflicts, on the one hand expressing its strong opposition to some of the provisions in the

European fusion sustained

Brussels

Funds to be devoted to Europe's research effort in controlled thermonuclear fusion were increased from 900 to about 1.400 million European Currency Units (1 ECU = £0.56) at a meeting of the EEC's science ministers in Brussels last week. JET, the Joint European Torus, which is being built at Culham in the United Kingdom will have a 319 million ECU budget for 1982--86 when the construction phase will be completed. European Community finance for other European fusion research has been set at 301 million ECU. This, with the contributions from national research budgets, will bring European fusion spending up to 1,400 million ECU over the next five years. The contribution from the EEC's budget for JET alone is thus increased from 195 to 319 million ECU.

In fact, most details of the fusion programme had been worked out well before the ministerial council; however, the British did their best to cut down support for general fusion research to 280 million ECU, a long way from the 325 million ECU originally requested by the European Commission, by arguing that the Commission had overestimated the speed of new developments. The European Commission calculated that 80 scientists would be needed to work on planning NET, the Next European Torus, and that accounted for much of the increased budget. But UK officials thought that because countries such as the United States or Japan were making do with 50 planners, the EEC could also do with fewer. The 301 million ECU finally agreed are supplemented by national contributions for projects involving tokamaks smaller than JET and other types of plasma confinement systems. The timetable for JET

foresees its tokamak in operation by the end of 1986.

At the press conference held by Philippe Maystadt, the Belgian science minister. who chaired the council, speculation was aroused by the omission of further international cooperation with the Soviet Union. Cooperation with Japan and the United States is to be strengthened during the new programme, he said. All four fusion "powers" are, however, cooperating with the International Atomic Energy Agency in Vienna on the Intor project, a post-JET tokamak, originally proposed by the Soviet Union. Cooperation among the Western powers is taking place simultaneously within the International Energy Agency in Paris and this may now be strengthened. The European Commission refutes the idea that an iron curtain is to be drawn across fusion research as a result of the present political tensions, since it is a field which has traditionally been kept outside East-West politics.

The meeting also approved a research programme on raw materials for 54 million ECU which covers a wide variety of different research projects including research in metals and minerals, wood, the recycling of non-ferrous metals and ceramics.

The rest of the meeting was devoted to discussing two policy papers on the reorganisation of European research cooperation resulting from the meeting held last November. Among the ideas now being given serious consideration is the setting up of a Perception and Assessment Committee, which would bring together about twenty senior members to analyse the trends of science technology and guide EEC projects to counterbalance any deficiencies.

Jasper Becker

current draft of a proposed international treaty, on the other not wishing to provoke a split with other industrialized nations which, although expressing reservations, are less inclined to reject the treaty as it now stands.

Last Thursday, the US delegation presented the conference with a list of changes that it says must be made before it will sign the treaty. These include the abandonment of efforts to place limits on the amounts of different minerals that can be retrieved from the sea bed, a revised voting procedure that would give the United States greater influence over the actions of an international authority established to regulate deep-sea mining, and dropping the requirement that US mining companies should provide Third World consortia with the latest mining equipment.

At the beginning of what is expected to be the final round of negotiations started in the early 1970s, several non-aligned countries indicated that it was too late to reopen discussion on topics which, they claimed, had been settled at earlier negotiating sessions. These countries said they would proceed with drawing up a treaty, leaving the United States to decide whether or not it wants to sign.

Behind the rhetoric, however, the real question is how far each side is prepared to compromise. A treaty without a US signature, or US financial support for the regulatory institutions which would be set up, would be of limited impact. From the American point of view, some prospective mining companies are concerned that they might be unable to raise credit from multinational banks if the treaty went ahead without a US signature, while several industrialized countries - including France, Britain and West Germany - are reluctant to jeopardize their trading relationships with Third World nations by providing too much support for the United States.

David Dickson

Polish higher education

Reform reversed

Research projects in Poland are now to be "rechecked" to assess their usefulness to the national economy, according to Dr Benon Miskiewicz, the new Minister of Science, Higher Education and Technology. Furthermore, the system for appointing assistant lecturers will be modified, and new principles worked out for the employment of graduates.

Dr Miskiewicz was addressing a meeting of lecturers from higher educational institutes subordinate to his ministry. The gathering did not include medical schools or specialist bodies such as the Mining and Metallurgy Institute of Krakow which, last September, had been a principal focus of resistance to attempts by the then minister, Mr Jerzy Nawrocki, to renege on the academic autonomy which, it had been

promised, would be incorporated into the new higher education bill. This bill, much delayed by the imposition of martial law, is now apparently undergoing redrafting and modification before going forward to the Seim (parliament).

Dr Miskiewicz's emphasis on the "usefulness" of research suggests a reemphasis of the "problem-orientated" research structure introduced in 1973 by which all research had to be associated with a specific problem of the national economy. This led to an unseemly scramble by researchers to find a "problem" rated as highly as possible by the planners. During the open press debates last summer on the future of Polish science, it was generally admitted that the "problem" structure had failed and should be replaced by some type of direct contact with industry.

By tacitly repudiating such proposals, Dr Miskiewicz was apparently trying to dissociate his ministry from such proposed reforms. During the past three months, there has been some high-level disagreement as to who was primarily responsible for the outspokenness of the academic community - the Academy of Sciences or the universities. Talking to the editorial board of the weekly magazine Polityka last January, Deputy Premier Mieczyslaw Rakowski said that the breaking point had come when the Conference of University Rectors had demanded a voice in the appointment of the Minister of Science, Higher Education and Technology. The conference, established in September 1980, was always regarded with suspicion by the government, and shortly after the declaration of martial law it was dissolved - unlike the majority of organizations which were simply suspended.

Officials of the Ministry of Science, Higher Education and Technology, however, blame the Academy of Sciences, whose institutes, they say, harboured dissidents who had been banned from university teaching. The proposed new law on the academy, which would have made the academic secretary responsible only to his fellow members and not, as at present, to the prime minister, has been abandoned. The limited autonomy which the academy has until exercised over its research budget, is likely to be reduced even further.

The authorities, however, seem unwilling to risk any further direct confrontation with the academy. A recent meeting of the presidium of the academy was addressed by Dr Hieronim Kubiak, the Communist Party spokesman on higher education, who is reputed to be the most liberal member of the Politburo. Dr Kubiak warned the academicians against pointless confrontation, stressing that the intelligentsia should "advocate a Poland which is possible". Nevertheless, he said, if there are reforms which are actually possible at present, one should support them openly and actively. Vera Rich

Industrial lure

New York

As five university presidents prepare to discuss the growing connection between universities and commercial interests in recombinant DNA research (Nature 4 March, p.1), private biotechnology firms continue to have an increasing impact. This week, two top academics decided to give up basic research posts to join private companies.

Dr Walter Gilbert, 1980 Nobel prize winner in chemistry, is to leave his American Cancer Society professorship at Harvard University as of June to retain his position as executive officer of Biogen, the Geneva- and now Cambridge-based biotechnology firm.

Dr Gilbert has had a two-year leave of absence from Harvard to fulfil his position as chairman of the board of directors of Biogen. His hopes of permanently maintaining both his laboratory at Harvard and his position at Biogen ran into unresolvable problems with the Harvard administration. He will, however, remain a senior associate of the university for two more years. His Harvard laboratory will continue to operate during this time.

His full-time commitment to Biogen comes at a time when the company's new American laboratory in Cambridge, Massachusetts has recently adopted another academic, Dr Richard Flavell from the National Institute of Medical Research at Mill Hill, as research director. Dr Flavell is officially on two-year leave of absence from Mill Hill.

Another top American academic to move into the private sector, Dr Edward Scolnick, chief of the laboratory of tumour virus genetics at the National Cancer Institute, has decided to become Merck, Sharpe and Dohme's executive director of virus and cell biology.

Michael Stein

UK budget

A gentle push

The British government's enthusiasm for new technologies, and its lack of enthusiams for the universities, was confirmed in last week's budget. While the universities can expect little respite from the austerity promised a year ago, the development of new technologies in industry is to receive a modest boost. British Telecom, which is to be allowed to increase its annual capital expenditure by a half over the next three years, is the chief beneficiary. But the Chancellor of the Exchequer has also promised the Department of Industry an extra £130 million for new technology in the next financial year, 1982-83.

British Telecom, which has recently improved its profits, will now be able to invest £2,300 million on capital projects in 1982-83, rising to nearly £3,000 million in 1984-85 mostly from its own resources.

British Telecom is now adapting its business to the liberalization announced by the government last October, the chief effect of which is to allow other companies to attach equipment to the telephone network. The government is, however, now apparently considering taking the liberalization a step further by selling off part of British Telecom. The Department of Industry would not comment last week on suggestions that a new telecommunications bill is being drafted for debate in the autumn. But ministers are discussing a document outlining several options for future telecommunications policy.

The Department of Industry is also in the throes of deciding how to spend its own windfall in last week's budget. Around £30 million of the £130 million promised for new technology projects this year will be spent on teletex, a superior type of telex. Competitors for the remaining £100 million include programmes to support the use of microprocessors in industry and research on flexible manufacturing systems, information technology and electronics.

The department's space budget, however, is likely to receive at least some of the extra money, possibly £5 million a year over the next three years. Judy Redfearn

Gene manipulation watchdog

Too smooth?

The uniquely British way of regulating research in genetic manipulation, the Genetic Manipulation Advisory Group (GMAG), may yet be a victim of its own success. The immediate difficulty is that its work seems now to have become so uncontentious that some of its sponsors and even some of its members are asking whether it should continue to exist. A decision one way or the other is likely by the end of the year, when the chairman of the group, Sir Robert Williams, and several of its members come to the end of their term of office.

Since GMAG three years ago adopted a method of qualitative risk assessment for categorizing experiments in genetic manipulation, at least its academic customers appear to have been satisfied with the informality of the procedures. Most academic work in the field appears to require only that laboratories should follow good microbiological practice or, alternatively, the lowest category of safety containment.

While notification of experiments is still legally required, this may be retrospective (in an annual report) for work carried out in open laboratories, while advance approval is no longer required for the

majority of experiments in the lowest catgegory of containment. Notifications of experiments in the second level of containment appear to amount to only about half a dozen a year.

For practical purposes, the burden of assessing the safety of experiments has been devolved from GMAG to laboratory safety committees. The bulk of GMAG's work seems now to consist of the assessment of safety arrangements in laboratories newly embarking on genetic manipulation and the drafting of guidance notes—a revised (and relaxed) procedure for dealing with manufacturing processes appeared only last week.

One substantial problem remaining is the systematic categorization of experiments involving viruses which are not covered by the regulations on the laboratory use of dangerous pathogens but which would involve hazards not now foreseen — oncogenic viruses for example. GMAG is wondering whether to set up a committee to look into this question. But the group has shied away from the field of human embryo transplants on the grounds that such work does not fall within the statutory definition of genetic manipulation and because its members do not have the necessary expertise.

Although both the Confederation of British Industry and the Trades Union Congress, which nominate members of the



group, still mutter that regulation of genetic manipulation might advantageously be transferred to the Health and Safety Executive, which has a statutory responsibility for safety at work, this view is no longer being strongly promoted.

Academic clients of GMAG, on the other hand, would prefer that responsibility should remain with the group, which has become the devil that they know. Academic investigators would also prefer that GMAG should continue in being so as to deal with whatever unforeseen problems may arise.

The most serious threat to the continued existence of GMAG seems to ennui, the lack of obvious challenge. Keeping the group alive is a modest public expense, making it a target for would-be economizers, but abolition would presumably require legislation — and parliamentary time is as scarce as money.

US electronics industry

Pooling effort

Washington

Faced with increasingly intense competition from the Japanese electronics industry, a number of large American computer companies are developing plans to pool a proportion of their research resources into a new venture, provisionally called Microelectronics and Computer Technology Enterprises (MCTE).

The idea is to achieve economies of scale for the companies, many of which invest up to 10 per cent of their annual revenues in research, by jointly funding research projects in such areas as materials science, computer-aided design, manufacturing processing and quality control.

Sixteen companies met in Florida last month to discuss plans for such a venture, which would have a research budget expected to rise eventually to about \$100 million a year. The meeting was also attended by representatives of the Department of Defense and the

UN assists India

New Delhi

The scheme developed by the United Nations Development Programme (UNDP) to encourage Indian scientists settled abroad to return to temporary work in India seems to be making progress. The TOKTEN scheme (Transfer of Know-how Through Expatriate Nationals) got under way early last year (see Nature 25 January 1981, p.343) and since then 32 Indian scientists from the United States, West Germany and Canada have visited India to work with industrial units and research institutions.

India has recently become alarmed at the extent of its "brain drain", especially among scientific personnel. Although there are no official figures, estimates place the number settled abroad at more than 100,000.

The TOKTEN scheme, already hailed as a success in Turkey, was introduced in India, with an initial contribution of \$1.1 million to encourage scientists settled abroad to give the benefit of their knowledge to their homeland. UNDP pays the fares of those who agree to visit India for a period of two or three weeks to help solve technical problems in sectors chosen by the government. The project, which is being implemented by the Council of Scientific and Industrial Research, has evoked a favourable response from both the Indian experts and the organizations with which they have worked, many of which have asked that these scientists should make a return visit or that more should come Sunil Saraf forward.

Massachusetts Institute of Technology, both of which have indicated their possible interest in collaborating with the joint research effort.

Pressure to find ways of linking the generic research needs of different companies in the computer industry has increased as the costs of research — both in real terms and as a proportion of revenues — has continued to rise.

A report published in 1979 by a panel of computer scientists established by the National Science Foundation suggested that a foundation should be established to raise funds from electronics companies and distribute them to university research departments for carrying out basic research.

As a step in this direction, the Californiabased Semiconductor Industries Association has established a Semiconductor Research Cooperative (ISRC) to act as a clearing house for companys interested in sponsoring university research. The idea of a separate foundation, however, has remained on the shelf.

Mr William Shaffer, spokesman for the Control Data Corporation which organized the Florida meeting, said last week that the ISRC initiative did not go far enough for the industry and was more limited in scope than the proposal which was discussed in Florida which would embrace not only a range of research activities designed to enhance the production of electronics and semiconductor components — an area in which the United States has lost considerable ground to Japan in recent years — but also provide training and other kinds of assistance to collaborating companies.

Computer manufacturers present at the meeting included Burroughs Corporation, Digital Equipment Corporation, Honeywell Incorporated, Sperry Corporation and Rand Corporation. Among the semiconductor producers were Advanced Micro Devices Incorporated. IBM had been invited to participate but did not attend.

The companies agreed to a general framework for a joint research venture that would embrace both basic and applied research. Such a joint venture will have to avoid conflict with strict American anti-trust laws, which have previously been quoted as a major impediment to collaborative research between different companies in the same industry. The need to "clarify" the application of anti-trust laws to research was one conclusion of an eighteen-month review of domestic policies affecting technological innovation carried out under President Carter; a subsequent report from the Department of Justice, published in November 1980, concluded that "in general, the closer the joint activity is to the basic research end of the research spectrum . . . the more likely it is to be acceptable under the anti-trust laws".

Control Data Corporation says that the outline of its proposals already presented

to the Federal Trade Commission has not met with any objections. The company is hoping that, by stressing the intention of MCTE to carry out research rather than produce electronics components, any potential conflict with the anti-trust laws will be minimized. **David Dickson**

UK-Romania cooperation

One-way street

Ruchares

The meeting earlier this month of the UK-Romanian Joint Commission on Trade and Technology produced a novel suggestion from the Romanian delegation: the establishment of long-term joint technological research projects. But the suggestion is unlikely to stimulate a positive response from the British side, since attempts at cooperation over the past decade have almost all become bogged down in bureaucracy and delay.

Even the one moderately viable project—the production under licence in Romania of BAC-111 aircraft—has run into difficulties; and hopes that the finished product might be exhibited at this year's Farnborough air show are rapidly fading.

Joint research and development ventures are impeded not simply by the currency problems endemic in all dealings with Eastern Europe but also by the reorganization of Romanian science during the seventies which has been specifically aimed at eliminating dependence on foreign licences and the development of a native technological base. According to Romanian planners, during the past five years, Romanian research has provided some 90 per cent of the new materials and more than 90 per cent of the new technologies put into production. This appears to leave little room for technological flow and exchange.

Romanian-British cooperation is formally of two kinds. Pure research, including exchange scholarships, is handled by an agreement between the Royal Society and the Academy of Romania. The restructuring of Romanian science has, however, reduced the academy's role.

The driving force in Romanian science is now the National Council for Science and Technology, headed by Dr Elena Ceaucescu, wife of the president, and the main research potential is concentrated in ten "Central Research Institutes" whose emphasis is largely on applications. At the height of the reforms, in the mid-1970s, it seemed that fundamental research might vanish altogether in Romania; now, however, 10 per cent of the science budget is allotted to "fundamental research".

Although at the academic level it has been possible over the past few years to arrange some cooperation projects — such as the exchange agreement between the Polytechnic University of Bucharest and the Polytechnic of Central London, the types of programmes that would normally fall within the competence of the joint commission are considerably harder to arrange. A joint seminar, held in Bucharest last May, on problems of energy saving in agriculture was — from the Romanian point of view — highly successful, but the British side is reluctant about a return visit, since they felt the "pooling" of knowledge came almost entirely from the British side.

Romanians involved in such ventures admit the disparity. But such one-side arrangements can never prove satisfactory in the long term to the "donor" partner, particularly when all procedural matters are hamstrung by bureaucracy. For, according to a law of 1974, any Romanian scientist contacted by a foreigner must report the matter to the relevant ministry. Although a number of prominent scientists say this regulation is a dead letter, the atmosphere of delay and obfuscation is hardly conducive to international cooperation.

Vera Rich

Bio-fund open

Those wishing to take advantage of the European Community's offer to support research and training in biomolecular engineering should think fast. Applications for research contracts are needed by 15 May, and for training contracts by July.

After a long and arduous gestation period, the EEC sponsorship of biomolecular engineering was looked on as something of a disappointment — "we came out with a mouse", said Vicomte Etienne Davignon, commissioner for research and development in Brussels (Nature 28 January, p.273).

But for all that, there is about 8 million European Currency Units (£4.5 million) available, and in the United Kingdom the Department of Industry shows signs of being anxious to alert UK scientists to the possibilities of obtaining EEC support for their work.

Research contracts are available for projects mainly related to agriculture and the safety of genetically manipulated organisms. Training contracts lasting 1 to 2 years from January 1983 cover a wider range of subjects including the development of cloning systems and gene transfer in agriculturally important plants. The commission invites submissions from "employed scientists (including those employed by industries) or unemployed scientists (who recently completed a doctoral thesis or postdoctoral assignment) who have demonstrated their capacity for high level scientific research". Applicants should approach the European Commission, but the Department of Industry will advise UK scientists how to apply. Charles Wenz

Congress tackles 1983 budget

Close vote on science budget a surprise

Washington

In the first congressional action on the research and development budget for the fiscal year 1983 submitted by President Reagan at the beginning of February, a subcommittee of the House of Representatives Science and Technology Committee agreed by one vote last Thursday to recommend that the budget for the National Science Foundation (NSF) be increased by \$30 million over the President's request to a total of \$1,099.5 million.

The subcommittee left untouched the proposal to spend \$1,052.3 million on "research and related activities" sponsored by NSF, although it has suggested some reshuffling between categories. For example, it suggests cutting from 9.5 to 5.6 per cent the proposed increase in funding for mathematical and physical sciences, and that biological, behavioural and social sciences should grow by 14.2 rather than 6 per cent, particularly in the light of the major cuts in social science research last year.

The subcommittee has also proposed transferring some of the funds for research to support the International Institute for Applied Systems Analysis in Vienna. However, the Reagan Administration's decision to eliminate its contribution to the institute, originally taken last year but since reconsidered, is said to have been made final at a meeting of the National Security Council last week.

The main difference between the subcommittee's and the Administration's proposals are over NSF support for science education. Whereas the Reagan Administration, committed to phasing out such activities within NSF, has proposed a budget of \$15.0 million in 1983 — down from \$70.7 million in 1981 — the members of the subcommittee have registered their opposition by adding another \$30 million.

It was primarily this decision that led the Republican members of the subcommittee, as well as newly-elected Democrat Dave McCurdy of Oklahoma, to vote against the proposed increases. Disagreement over the science funds is also expected to generate another close vote in the full science committee when the budget authorization bill comes up for approval.

The closeness of the vote, especially among the members of what is generally considered to be one of the most proscience subcommittees in Congress, suggests that the final figures for government support of research and development

next year are likely to be close to those submitted by President Reagan, since Democrats seem reluctant to ask for major increases in the science budget and Republicans are reluctant to demand major decreases.

According to a preliminary analysis of the Administration's budget request released last week by the American Association for the Advancement of Science (AAAS), the net result of the request would be to increase research and development spending by 2.2 per cent in real terms over 1981 to \$44,400 million.

Within this total, basic research will increase from \$5,100 million in 1981 to \$5,900 million in 1983, an increase of 14.9 per cent in current dollars, but a decrease of 1.3 per cent in constant dollars. The AAAS analysis demonstrates clearly that the expansion of research budgets is almost entirely explained by the growth of military-supported research.

Much grousing has been heard on Capitol Hill over the past few weeks as different committees have held hearings on the Administration's proposed budget. Some of the loudest have come from members of the space science community where, although major projects such as the Galileo mission to Jupiter have retained support in an overall budget that increases by 28 per cent between 1981 and 1983, several research programmes will still have to be terminated.

Quoting examples such as the decision to close the lunar curatorial facility, the main centre for the analysis of Moon samples and cosmic dust collected by the Apollo missions, as well as to shut down the transmission and reception of data from Pioneers 10 and 11, launched in the mid-1970s and still sending data from deep space, the planetary sciences division of the American Astronomical Society has released a statement claiming that the Reagan budget would devastate planetary research at the National Aeronautics and Space Agency.

A strong critic of the Administration's proposals for energy research has been Dr John Deutch, who was director of the Department of Energy's Office of Energy Research for the first two years of the Carter Administration, and is now dean of science at the Massachusetts Institute of Technology. Dr Deutch told a Senate subcommittee last week that the proposed cuts in energy research and development supported by the federal government would have a damaging effect on the nation's future energy supply. The private sector could not be expected to undertake all necessary research and development in energy, said Dr Deutch: "a policy based on this assumption places our technical future in energy technology at risk".

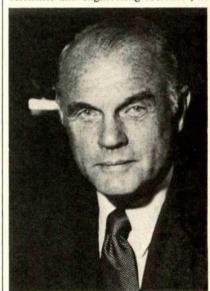
Whereas the Administration is planning to cut support for coal research and development from \$500 million in 1982 to \$84 million next year, Dr Deutch suggested it should be raised to \$625 million. In contrast, rather than asking for \$557 million for the support of breeder reactor systems research, he suggested cutting this back by \$125 million and abandoning the proposed liquid metal fast breeder reactor at Clinch River in Tennessee, which he described as "unneeded and technically obsolete".

The most important factor determining the final outcome of the science budget will be the broader political debate on how much of a federal deficit the nation's economy can tolerate. If Congress decides that the proposed deficit is too large, but is reluctant to cut back on demands for increased military spending (or to increase tax revenues), then it will be looking for more cuts in the civilian budget. Whether this will be necessary depends on deliberations within the budget committees of the Senate and the House of Representatives that will be taking place over the next two David Dickson months.

Glenn speaks out

One of the strongest critics of the Administration's policies towards the support of research and development is Senator John Glenn of Ohio who, like Senator Harrison (Jack) Schmitt, the chairman of the Commerce Committee's science and technology subcommittee, was a member of NASA's early astronaut team.

Senator Glenn has been addressing scientific and engineering societies, in



Glenn - from spaceship to White House?

particular advocating strong unilateral measures to prevent the proliferation of nuclear weapons through controls on nuclear exports. He is also expected to be a candidate for the Democrat presidential nomination in 1984, offering an alternative vision of liberalism to those of Senator Edward Kennedy and former vice-president Walter Mondale.

Professional pride

SIR — On page 542 of your issue of 18 February you inveigh against the "deceit" of groups of professional people who combine to campaign against nuclear weapons. On page 545 we read ". . . the Pentagon's Defense Science Board, chaired by Dr John Deutsch of the Massachusetts Institute of Technology. recommended a start on the production of binary weapons and that the Department of Defense should prepare for a major increase in its chemical warfare programmes". I do not recall having seen in your editorial columns any denunciation of this kind of professional activity. Apparently it is all right for groups of bought scientists, operating mainly in secret, to promote the development of the instruments of mass murder, but somehow deceitful for groups of free scientists, meeting in public, to take the contrary stance.

J.R.S. FINCHAM

Department of Genetics, University of Edinburgh, Edinburgh, UK

Protein evolution

SIR — Hoyle and Wickramasinghe believe, probably correctly, that the age of the Earth is not great enough to have given time for chance mutations to produce enzymes specified at every point in a chain of, say, 200 amino acid units. Jukes (Nature 18 February 1982, p.548) replies that proteins evolve by elongation of small polypeptides. This reply is insufficient if the elongation process must still produce a unique arrangement of amino acid units of the chain length of the final enzyme.

The size of the initial polypeptide is irrelevant. The essential point is (see also Nature 3 December 1981, p.396) that enzymatic activity of a particular kind can arise from quite short lengths of polypeptide chain, for example six amino acid units. The rest of the chain, if present, may gain significance in the course of evolution, and must certainly have the property of not folding so as to enclose the active part of the chain. The presence of life on this planet does not, however, depend, as Hoyle supposes, on the emergence of the complete chain, specified point by point. The minimal length required for a particular enzymatic activity would not require Hoyle's endless aeons to appear by chance; it would be already present in the primitive ocean. A.E. ROUT British National Oil Corporation

(Trading) Ltd, London W1, UK

A wing and a prayer

Sig — Is Jukes a secret believer, or merely an unwary fox in among the fundamentalist chickens? The ancestral relationship he implies between the biplane and the Boeing 747 (Nature 18 February, p.548) is one of design, not of descent. This is a striking counterpart of the creation process. Tragically, God's Concorde has fallen a long way since his maiden bite at the evolutionary tree, and even American jumbos finally land up in (we hope) US junkyards. Pigs might, but they have the good sense not to try. C. DARNBROUGH The University of Glasgow, Glasgow, UK

Electricity costs

Sir - Professor Fremlin (Nature 1 October 1981, p.332) wonders why the retail price index is used to correct for inflation. The short answer is given by the Central Electricity Generating Board's (CEGB's) 1980-81 report, paragraph 168. "The cost per kWh of fuel. rose by 18.6 per cent (between 1979-80 and 1980-81). This was higher than the general inflation rate (a 16.3 per cent increase in the RPI) for a number of reasons". RPI is used almost universally as a measure of the general inflation rate. It also favours nuclear costs.

I have previously dealt1 fully with the question of interest rates for comparison purposes, and if Professor Fremlin wants to discover an investment giving a steady 5 per cent above inflation rate I suggest he keeps an eye on his electricity bills, because they will have to cover a real 5 per cent interest on the funds being built up for decommissioning etc., by CEGB.

As far as accuracy is concerned, CEGB cost figures are given to 1 in 10,000, so my 1 per cent accuracy in the tables of my letter (see Nature 27 August 1981, p.791) hardly requires a probable error. Where "estimated minimum (or probable) corrections" are given, the 'error'' arises from the refusal to release available accurate information and its magnitude is known only to CEGB.

On future action, until Professor Fremlin is prepared to take real account of the work of Gerald Leach and his colleagues2, I cannot take seriously such ex cathedra statements as his last sentence, "As of now it (nuclear power) is the only means capable of the scale of output needed".

I accept that cost ratios in 25 years' time are far more uncertain that the differences between my figures and those of CEGB. (Although it seems that there is considerably more uncertainty on nuclear than on coal costs.) The importance of the calculations of present costs is that CEGB claimed to be able to produce cheaper electricity by building new nuclear power stations immediately (Heysham II and the Sizewell PWR) even though they were not needed to satisfy demand. It has now been demonstrated1 that this claim was entirely dependent on the assumption of a massive (36 per cent) increase in the real cost of coal to CEGB over the six years of the construction period (1980-86). This assumption was very effectively camouflaged in the board's documents and this is not surprising since it was made at almost exactly the same time as the "understanding" was entered into with the National Coal Board which practically guarantees that the real cost of coal to CEGB will not rise at all between 1980 and 1985.

In that case building Heysham II will certainly result in dearer electricity than if it had not been built, but instead the large overcapacity of coal-fired stations, which are at present being prematurely retired, had continued in operation.

Building unnecessary expensive new power stations may not bankrupt CEGB, but it will not help industry get cheaper electricity.

J.W. JEFFERY

Birkbeck College. University of London, UK

- Jeffery, J.W. Energy Policy 10, No. 2 (in the press)
 Leach, G. et al. IIED Sci. Rev. (1979).

PCBs in rice oil

Sig — In March 1979, patients suffering from chloracne began appearing on the west coast of Taiwan. By October the disease had reached epidemic proportions, affecting over 1,100 people in the T'aichung and Changhua prefectures.

The exposure factor common to these patients was the consumption of rice oil produced by the same rice oil processing company situated in Changhua prefecture. Investigation of the manufacturing process identified a leak in a heat exchanger, which resulted in oil contamination with Kanechlor 400, a 48 per cent chlorinated biphenyl. In addition to symptoms associated with chloracne, clinical signs included respiratory symptoms, decrease in conduction velocity in peripheral sensory nerves, diminished growth in young children, and four reported cases of pigmented skin in newborns, all of whom died within a year of birth.

The same type of mass rice oil contamination was reported in western Japan in 19681, affecting more than one thousand victims. These were cases of direct consumption of high doses of polychlorinated biphenyls. The use of these substances was banned in Taiwan in 1972, but that did not prevent this recurrence, which is evidence that Kanechlor 400 is still being used in the heat exchange process during the production of oil for human consumption. This recurrence should serve as a warning to those Asiatic riceproducing countries which still use these oil production systems.

WILLIAM Y.B. CHANG University of Michigan, Ann Arbor, Michigan, USA

1. World Health Organization Envir. Hlth Criteria 2 (1976).

Holmes' lives on

SIR - What's this nonsense about Holmes' Principles of Physical Geology being "long out of print"? ("What Makes a Good Textbook"; Nature 11 February, p.459.) This work has never been out of print. A comprehensively revised third edition was prepared by Sir Arthur's widow Doris (herself a fine geologist in her own right) and published in a revised format in 1978. The book has, as you say, been a great success both critically and commercially. It continues to be so after nearly 40 years of uninterrupted availability. DOMINIC RECALDIN Thomas Nelson & Sons Ltd, Walton-on-Thames, Surrey, UK

An end to creation?

SiR — Why do you continue to publish letters on creationism? I am reasonably confident that you would not publish arguments on whether the Earth is flat or whether epilepsy is caused by demoniacal possession.

Of course, scientists are entitled to their private superstitions; but they have no place in a supposedly serious journal. Pray let us see those long overdue words: "This correspondence is now closed.'

M. HAMMERTON

Department of Psychology, University of Newcastle upon Tyne, UK

Science on the Space Shuttle

W.M. Neupert, P.M. Banks, G.E. Brueckner, E.G. Chipman, J. Cowles, J.A.M McDonnell, R. Novick, S. Ollendorf, S.D. Shawhan, J.J. Triolo & J.L. Weinberg

THE third flight of the Space Shuttle, chiefly intended to test the orbiting portion of the Shuttle in extreme thermal conditions in space, nevertheless carries a scientific payload. The investigations will demonstrate the Shuttle's capability for research in space plasma physics, solar physics, astronomy, life sciences and technology and will also determine the effects that the presence of the Orbiter may have on its immediate environment. The information to be gathered is thus the foundation for planning of future investigations with the Space Shuttle.

The scientific payload is designated OSS-1 because the programme was originally managed by the Office of Space Science (now the Office of Space Science and Applications) at NASA headquarters. Responsibility for development of the payload was assigned to the Goddard Space Flight Center. The manager for the scientific payload is Kenneth Kissin. The OSS-1 instruments will study the Orbiter's plasma environment and the propagation of an electron beam in space, record the Sun's UV and X-ray fluxes, observe the zodiacal light and Milky Way, record interplanetary dust particle impacts, operate a sophisticated heat pipe system and also grow plants in the Orbiter's cabin. Demonstrating the Orbiter's research capability demands accurate control of the Orbiter's orientation during solar observations and use of a Remote Manipulator System to carry a package of sensors that will map charged particle and

Werner Neupert is OSS-1 Mission Scientist and Head of the Solar Plasmas Branch, Laboratory for Astronomy and Solar Physics, at NASA Goddard Space Flight Center, Greenbelt, Maryland; Peter Banks, Principal Investigator of the Vehicle Charging and Potential Experiment, is Adjunct Professor Physics at Utah State University, Logan, Utah, and Professor of Electrical Engineering at Stanford University, Stanford, California; Guenter Brueckner, Principal Investigator of the Solar Ultraviolet Irradiance Monitor, is Head of the Solar Physics Branch at the Naval Research Laboratory, Washington DC; Eric Chipman is Program Scientist for the OSS-1 mission at NASA Headquarters, Washington DC; Joe Cowles, Principal Investigator of the Plant Lignification Experiment is Professor of Biology, University of Houston, Houston, Texas; J.A.M. McDonnell is Principal Investigator of the Microfoil Abrasion Experiment and Reader in Space Sciences at the University of Kent, Canterbury, UK; Robert Novick, Principal Investigator of the Solar Flare X-ray Polarimeter, is Professor of Physics, Columbia University, New York; Stanford Ollendorf, Principal Investigator of the Thermal Canister Experiment is Head, Spacecraft Component Development and Analysis Section, Goddard Space Flight Center; Stanley Shawhan, Principal Investigator of the Plasma Diagnostics Package, is Professor of Physics, University of Iowa, Iowa City, lowa; Jack Triolo, Principal Investigator of the Con tamination Monitor Package is in the Spacecraft Component Development and Analysis Section, Goddard Space Flight Center, Greenbelt, Maryland; and J.L. Weinberg, Principal Investigator of the Shuttle Spacelab Induced Atmospheres Experiment, is Research Scientist and Director of the Space Astronomy Laboratory at the University of Florida, Gainesville, Florida.

magnetic and electric field distributions around the vehicle.

The same instruments will make sensitive measurements of how the Orbiter alters its environments by the emission of particles or electromagnetic fields. Although the spacecraft has been designed to minimize such effects some, such as propellant plumes from thruster engine firings or electromagnetic radiation from the electrical circuits, are unavoidable.

physical conditions that led to the formation of the aurorae, seen at high latitudes!

The Vehicle Charging and Potential Experiment is the first opportunity to make vehicle charging and electron beam studies from a manned spacecraft. In the first instance, measurements will be made of the effect of the natural ionospheric environment on the gross electrical characteristics of the Orbiter using two pairs of charge and current probes, situated on opposite corners of the pallet, which simulate both the electrically insulating and conducting portions of the Orbiter's surface, and with a Langmuir Probe-Spherical Retarding Potential Analyser located on the sill of the pallet.

Active electron emissions will be used to change the natural electrical balance to

The nine instrument packages on the third Shuttle flight should yield important data for space plasma physicists, astronomers, life scientists and engineers and also pave the way for future scientific payloads.

All but one of the OSS-1 instruments are mounted on an engineering model of the Spacelab pallet manufactured by the European Space Agency (Fig. 1). The instruments, pallet and the various subsystems for command, data handling, power and thermal control weigh 3,132 kg. The Plant Lignification Experiment is located in mid-deck lockers of the Orbiter cabin and two OSS-1 tape recorders and two control panels are located in the aft flight deck on the upper level of the cabin. Most internal experiment operations will be carried out via commands from the investigators located at the Payload Operations Control Center at Johnson Space Center in Houston, Texas.

On the third Shuttle flight, scheduled for late March, the Orbiter will be placed in a circular orbit of 241 km with an inclination of 38°. During the seven-day flight, the Orbiter will be held in several attitudes as part of the mission's thermal test objectives. The 28-hour long orientation of the Orbiter bay towards the Sun is suitable for solar observations. The planned 80-hour long period when the Orbiter will be maintained with its nose towards the Sun is a prime observing interval for the plasma physics investigations.

The nine experiments will now be described separately.

Electromagnetic environment

During the next decade, active electron and ion beam experiments in space will become an important tool for probing the environment above the Earth's atmosphere. This region contains plasma and magnetic fields of both solar and terrestrial origin. Hitherto, actively controlled charged particle experiments carried by rockets and unmanned satellites have been used to probe the structure of the Earth's magnetic field and to create the

determine how a charged Orbiter will affect electron beam and direct plasma measurements. The electron source (the Fast Pulse Electron Generator) emits a 100-mA beam of nearly monoenergetic 1 keV electrons. Pulses as short as 600 ns or as long as 109 s can be generated (Fig. 2a).

The Fast Pulse Electron Generator will also be used to investigate the effectiveness

Table 1 OSS-1 Plasma Diagnostics Package instrumentation and measurement ranges

●Low energy proton and electron differential energy analyser

Nonthermal electron and ion energy spectra and pitch angle distributions for particle energies between 2 and 50 keV

A.c. magnetic wave search coil sensor
 Magnetic fields with a frequency range of 10-30

●Total energetic electron fluxmeter

Electron fluxes between J_{0}^{9} and I_{0}^{14} electrons cm⁻² s⁻¹

●A.c. electric and electrostatic wave analysers Electric fields with a frequency range of 10 Hz-1 GHz

S-band field strength meter

•D.c. electrostatic double probe with spherical sensors

Electric fields in one axis from 2 mV m⁻¹ to 2 V m⁻¹

●D.c. triaxial fluxgate magnetometer Magnetic fields from 12 mG to 1.5 G

●Langmuir probe

Thermal electron densities between 10⁴ and 10⁷ cm⁻³

Density irregularities with 10 m-10 km scale size

Retarding potential analyser/Differential velocity probe

Ion number density from 10² to 10⁷ cm⁻³ Energy distribution function below 16 eV Directed ion velocities up to 15 km s⁻¹

•Ion mass spectrometer

Mass ranges of 1-64 AMU

Ion densities from 20 to 2×10^7 ions cm⁻³

●Pressure gauge

Ambient pressure from 10⁻³ to 10⁻⁷ torr

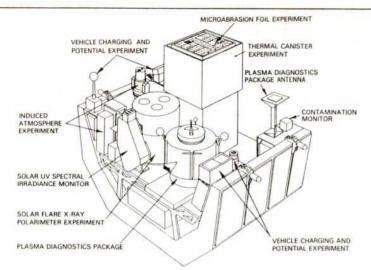


Fig. 1 OSS-1 instrumentation mounted on an engineering model pallet supplied by the European Space Agency. An additional OSS-1 experiment is carried in the mid-deck section of the Orbiter cabin.

of generating electromagnetic radiation by pulsing the electron beam at frequencies as high as 1 MHz. Several ground-based observatories will attempt to receive this radiation. Of particular interest is the extent to which VLF radiation can be generated using special electron beam modulation sequences. Detection will be attempted both at ground observatories and with VLF receivers aboard the recently launched Dynamics Explorer satellites.

Evidence will also be sought for a laboratory phenomenon termed beamplasma discharge, in which an electron beam energizes a plasma column which then emits intense optical radiation and radio waves (Fig. 2a). It is thought that the electron beam initially ionizes residual gas, producing a columnar plasma. As the plasma density builds up, the beam creates electrostatic plasma waves which impart additional energy to the plasma, leading to further ionization and excitation and causing the column to emit optical and radio waves. In Earth-orbit, the different conditions may mean that beam plasma discharge does not occur - the gas density is less, the beam is not terminated by the metallic top of a chamber and the Orbiter moves the beam across magnetic field lines so that plasma may not build up. The Vehicle Charging and Potential Experiment will be used with the Plasma Diagnostics Package to tell whether beam plasma discharge is initiated by the beam from the orbiting electron generator (Fig. 26).

Ionosphere studies

The Earth's ionosphere can be studied by introducing perturbations such as chemical tracers, radio waves and particle beams and by creating plasma wakes around solid bodies. The objectives of the Plasma Diagnostics Package, an assembly of electromagnetic and particle sensors, are to assess the electromagnetic and plasma environment of the Orbiter, to study the interaction of the vehicle with the surrounding plasma, to test the capabilities

of the Remote Manipulator System and to carry out active beam-plasma experiments in conjunction with the Fast Pulse Electron Generator. Measurements will be made of electric and magnetic fields, plasma waves, energetic ions and electrons and plasma parameters — density, composition, temperature and directed velocity (see Table 1).

The Plasma Diagnostics Package will be operated both on the OSS-1 pallet and while deployed by the Remote Manipulator System. As the Plasma Diagnostics Package is moved in and around the Orbiter bay, measurements will be made of the ambient pressure and of the spectrum of electromagnetic interference generated by the Orbiter's electrical subsystems. The pressure profiles in time and in distance from the Orbiter are relevant for the design of instruments sensitive to gaseous contamination and those requiring low operating pressures. The sensitivity of wave receivers and of topside ionospheric sounders to be flown on future Spacelabs will be determined by the levels now measured, while measurements of electric fields and particles by the Plasma Diagnostics Package will provide an independent assessment of the charge condition of the Orbiter.

The Orbiter will move in the ionosphere, at supersonic velocity (Mach number 6 relative to the characteristic plasma sound speed or the ion acoustic sound speed2) and so will create a plasma wake that may be identified by plasma depletion, energization of particles and the creation of Alfven waves behind the Orbiter. Such processes are thought to be important consequences of the motion of other bodies through plasmas - for example, Alfvèn waves behind the jovian moon lo may accelerate the particles which cause decametric radio noise bursts3. Remote Manipulator System trajectories have been designed to move the Plasma Diagnostics Package through the wake boundary, thus providing direct observations of its Physical properties. When the package

flies again on the Spacelab-2 mission as a subsatellite, the wake will be examined out to 20 km behind the Orbiter⁴.

The combination of the Fast Pulse Electron Generator and the Plasma Diagnostics Package provides an opportunity to study the interactions of a beam of accelerated electrons with the ambient space environment (see Fig. 2b). Radio waves over a wide frequency range may be stimulated by both pulsed and continuous operation of the electron beam and measured with the Plasma Diagnostics Package. The two instruments will also operate together to investigate beamplasma interactions such as the beam plasma discharge. Similar experiments will be conducted with more intense beams on Spacelab-1 and on later missions5.

Solar ultraviolet radiation

Solar UV radiation in the spectral range 120-300 nm has an important role in the energy balance and photochemistry of the Earth's upper atmosphere. Molecular oxygen absorbs radiation at wavelengths below 242 nm, resulting in its dissociation into atomic oxygen; ozone is dissociated into molecular and atomic oxygen by UV radiation below 310 nm. These reactions

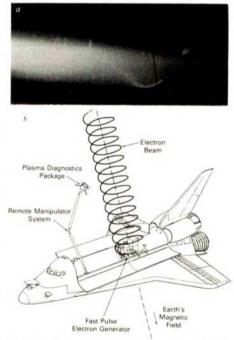


Fig. 2a, Photograph of light emission resulting from the interaction of electrons generated by Fast Pulse Electron Generator with residual atmosphere in a space simulation chamber at the Johnson Space Center. The column of luminosity enclosing the helical path of the primary electron beam is due to the occurrence of a beam plasma discharge for the conditions pertaining to this particular operation of the electron generator. b, Scheme for the joint Fast Pulse Electron Generator and Plasma Diagnostics Package operations. As the electron beam is emitted along some angle to the Earth's magnetic field, the Remote Manipulator System sweeps the Plasma Diagnostics Package back and forth across the beam region to make measurements of plasma fields and waves and of the energy distributions of electrons and ions.

take place at altitudes from 30 to 160 km in the Earth's atmosphere. Ozone is formed from molecular and atomic oxygen by a catalytic reaction which involves trace species such as NO, NO, or Cl. Quantitative description of these reactions requires precise knowledge of the absolute amount of solar UV radiation as a function of wavelength. Although many attempts have been made to measure this quantity, large experimental discrepancies still exist⁶ (Fig. 3). The objective of this first flight of the Solar Ultraviolet Spectral Irradiance Monitor experiment is to establish a more accurate base of solar UV irradiance measurements with an absolute error of 10 per cent or less over the wavelength region 120-400 nm. During the STS-3 flight, it will accumulate approximately 20 hours of solar measurements, compared with 5 minutes during a typical sounding rocket flight.

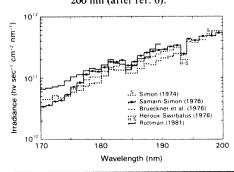
Calibration is important. The instrument carries two independent spectrometers and an in-flight calibration light source which allows tracking of any sensitivity change due to vibration at liftoff or contamination during flight. Its seven detectors will allow cross-checks of possible detector changes. It can be operated in broadband (5 nm) or narrow band (0.15 nm) modes over the wavelength region 120-400 nm. During 17 orbits amounting to approximately 28 hours, the bay of the Orbiter will be pointed to the Sun by the crew, using Sun sensors mounted on the Solar Ultraviolet Irradiance Monitor and measurements of solar intensities will be interleaved with periodic in-flight calibrations7.

The measurement of the solar UV irradiance on OSS-1 is only the first step of a programme to measure the variability of the solar UV radiation over an 11-year solar cycle. This variability has been estimated to be less than 20 per cent in the 170-210 nm region and less than 2 per cent in the 210-300 nm region⁸, and Fig. 3 shows that a decisive improvement of accuracy is needed if the Sun's UV variability is to be determined.

Solar flares

Although it is known that there are highenergy electrons in the Sun's atmosphere during solar flares, fundamental questions remain about the nature and location of the

Fig. 3 Recent measurements of solar UV irradiance in the spectral region between 170 and 200 nm (after ref. 6).



mechanisms by which the electrons are energized and their energy dissipated. It is, however, believed that a flux of magnetic energy from the solar interior provides the energy for these events, and that interactions of the accelerated particles with the Sun's atmosphere dissipate this energy, leading to observable flare phenomena including X-ray emission. Similar bursts of energy observed by the Einstein Observatory from other stellar objects have established that flares are relatively common. Hard X rays emitted during flares carry unique information about the motion of the electrons producing the radiation9. A definitive observation of the state of polarization of the radiation could provide important data to test theoretical models of the flare phenomenon.

The Solar Flare X-ray Polarimeter on OSS-1 aims to observe flare X rays emitted between 5 and 30 keV and to measure their polarization as a function of time and photon energy. The instrument uses blocks of metallic lithium surrounded by xenonfilled proportional counters as detectors. If polarized, the incident X rays will be scattered preferentially by the lithium into directions normal to the plane of the electric vector of the incoming radiation. To avoid instrumental effects that have plagued previous measurements, the instrument uses three independent sets of scattering blocks and detectors, with each unit rotated by 120° with respect to the other two about a line passing through the Sun. A minimum of two units is necessary to determine the magnitude and orientation of polarization; the use of a third provides redundancy and increased effective area10.

The instrument will be aimed at the Sun by orienting the entire bay of the Orbiter and it is planned to maintain the Orbiter in this orientation for approximately 28 hours. Solar flares occur only sporadically on the Sun, so that the observation of flare emission is not assured. However, the instrument has sufficient sensitivity that even a small event can yield a usable signal. Such events can be expected to occur about once a day on average.

Zodiacal light

The zodiacal light arises from sunlight scattered or absorbed by interplanetary dust particles, the characteristics of which have been partly defined by rockets and unmanned Earth-orbiting satellites11. Observations of colour, polarization and angular dependence are needed to determine dust particle size and composition¹². Unfortunately, accurate data are scarce. Thus, polarization results at different wavelengths are often combined. There are some observations of brightness, but few of polarization and colour in regions off the ecliptic, closer than 30° to the Sun and near the anti-solar point — the regions which contain the most information on the dust. The Shuttle Spacelab Induced Atmosphere Experiment

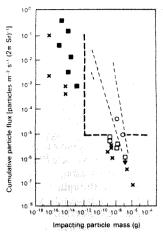


Fig. 4 Measurements of near-Earth microparticle fluxes by various techniques: , impact plasma sensors; ×, capacitor discharge sensors; O recovered surface examination; , perforation of pressurized cylinders; dashed lines, models derived from microphone sensors. The area to the upper right of the heavy dashed line is the sampling regime of the Microabrasion Foil Experiment on OSS-1. The cumulative particle flux is the flux of particles above a specific mass. Adapted from ref. 14.

will provide observations to determine more precisely the position of maximum concentration of interplanetary dust and will search for evidence of different particle characteristics for dust near the Sun.

Although astronomical observations from space avoid the effects of the Earth's atmosphere, the observing platform releases particulates which may form a local contaminant cloud that will also scatter sunlight into the detector. The presence of such an induced atmosphere can be established by comparing observations made when the spacecraft is in sunlight and in the Earth's shadow. The photopolarimeter system on OSS-1 will measure the light scattered from any local cloud of particulates and will record the brightness, polarization, colour and angular dependence of the diffuse astronomical background (zodiacal light and background starlight) at visual and near IR wavelengths.

The instrumentation to be used is the spare unit of the Skylab S073 photopolarimeter and bore-sighted 16 mm camera. The system, which includes an optical train, optical filters for 10 wavelength bands between 400 and 820 nm, a polarization analyser, a brightness calibration source and a photoelectric sensor, can carry out observing sequences according to pre-programmed routines. Although the instrument was operated on Skylab, difficulties with the airlock precluded observations closer than 80° to the Sun. A new gimbal mount has been designed for this mission, allowing the instruments to be scanned in a vertical plane running fore and aft along the Orbiter axis. The instrument will view the entire sky between 20° and 120° from the Sun. Limited observations will also be possible at larger angles.

Interplanetary dust

Direct sampling of interplanetary particulate matter has been carried out from balloons, U-2 airplanes, rockets, Earth-orbiting spacecraft13. Remote measurements have been made with sensors in Earth orbit and interplanetary space14. Even impact craters in lunar samples have provided valuable information. A wide range of particle masses, down to 10-15g have been observed, with the cumulative particle flux increasing strongly with decreasing mass (Fig. 4). While comets are thought to be the most likely source of interplanetary dust, direct measurement of particle composition could discriminate between cometary and asteroidal sources for the dust.

The Microabrasion Foil Experiment aims to measure the high velocity microparticle flux in near-Earth orbit, for particle masses greater than 10⁻¹²g, to investigate the density distributions of the impacting particles and to study their chemical properties by analysis of residues remaining in the impact craters. The sensor, attached to the thermal blanket on top of the thermal canister experiment, consists of approximately 1 m² of 5µm thick aluminium foil bonded to a gold-coated brass support mesh bonded to a Kapton sheet to form a double layer detector.

Differing types of impacts may be recorded. A particle of mass < 10⁻¹²g will form a hypervelocity impact crater on the foil surface, without penetrating the foil. More massive particles will penetrate the foil forming a characteristic 'penetration profile', dependent on the particle's incident velocity. Particles penetrating the foil may survive intact to produce a single impact crater on the rear Kapton sheet, or the particle may split into fragments and produce a corresponding number of impact craters on the sheet. Low density "fluffy" particles readily fragment

whereas high density iron or stony micrometeorites survive almost intact.

Unlike the other experiments, the Microabrasion Foil Experiment is passive. Post-flight measurements of the craters with scanning electron microscopy and energy dispersive X-ray microprobe analysis of residues will provide information on elemental composition, density and shape, and thus on the origin of these particles.

Plant lignification

Although few plants have been grown in space, Russian experiments have demonstrated that near-zero gravity disorients root and shoot growth, enhances plant sensitivity to substrate moisture conditions and generally results in a high mortality rate. However, little is known about the physiological changes that occur. Understanding of gravity's effects on plant growth and metabolism will provide an insight into plant physiology and aid development of an effective biological life support system.

After cellulose, lignin is the most abundant carbon compound in plants and provides both their strength and form. As gravity is believed to be a primary controlling stimulus for lignification the Plant Lignification Experiment will evaluate how near zero gravity affects the quantity and rate of lignin formation in different plant species during early stages of development.

Of the major groups of higher plants the gymnosperms will be represented by slash pine, the monocotyledonous angiosperm by oat, and the dicotyledonous angiosperm by the mung bean. All are compact species that may be grown in the limited space and relatively low light levels provided by the compact flight Plant Growth Unit. Oat and mung bean seeds and young pine seedlings will be planted in the growth chambers (Fig

5) before launch so that most seedling development will take place in a weightless environment. Electronics for controlling and monitoring temperature and light cycles are incorporated into the Plant Growth Unit. At the latest convenient time (about 7 hours before launch) the unit will be carried on board and installed in a middeck locker of the Orbiter cabin, where it will remain throughout the flight.

On landing, the Plant Growth Unit will immediately be removed from the Shuttle, the plants photographed, and the gaseous atmosphere of the plant chambers analysed. The seedlings will then be removed and analysed for lignin content.

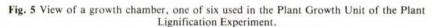
Control experiments, with the plants growing in a 1-g environment, will be conducted after the flight using the flight hardware and flight environmental data. Lignin data from the flight and control plants will be compared for patterns of lignin deposition to assess whether lignin is reduced in plants grown in zero gravity.

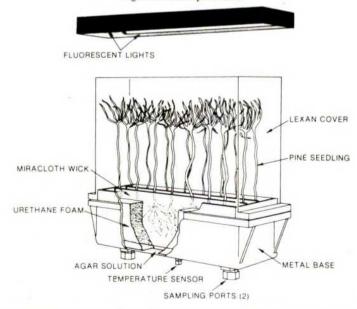
Because the reduced flight time of the previous Orbiter flight prevented completion of the Heflex (plant) Bioengineering Test that it carried, this experiment has been added to the current flight and is also carried in the Orbiter cabin. The test is being conducted specifically to determine the optimal soil moisture conditions for germination and growth of the sunflower *Helianthus annus* in near zero gravity. It is a prelude to a plant growth experiment to be conducted on the first Spacelab mission to be flown on the Shuttle in 1983.

Thermal canister

The long-term use of Space Shuttle means that many scientific and technical investigations can be performed in the Orbiter bay. However, the extreme thermal environmental conditions ranging from equivalent sink temperatures of + 100°C in full Sun, to -100°C, in shadow may cause problems. In the past such conditions were accommodated using coatings, insulation and heaters. With the Shuttle, an instrument designed for one set of conditions may have to survive in an entirely different environment if flown again with different orbit attitudes. If a thermal enclosure were provided which decoupled instruments from the wide extremes in external tempeature whilst maintaining them in a benign environment, simpler thermal designs for instruments, with limited maintenance between flights, might be realized.

To this end the Thermal Canister Experiment aims to determine whether a device using controllable heat pipes could maintain simulated instruments at several selectable temperature levels in zero gravity, and under widely varying internal and external thermal loads. It is hoped to demonstrate ±3°C temperature stability at various control points in the canister while dissipating up to 400 W in cold Orbiter attitudes (bay away from the Sun)





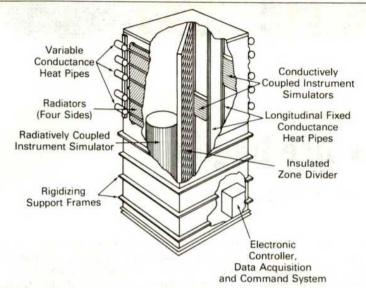


Fig. 6 Cutaway view of the Thermal Canister Experiment showing controllable heat pipes. In flight conditions, the lower half of the unit is covered with multi-layer insulation to provide a measure of isolation from temperature extremes in the Orbiter bay.

and 100 W hot (bay towards the Sun) conditions.

The Thermal Canister Experiment (Fig. 6) consists of a rectangular enclosure 3m high × 1m long × 1m wide with its aluminum sides equalized in temperature by a system of longitudinal fixed conductance heat pipes. These heat pipes collect the thermal energy dissipated internally by electrical heaters simulating instruments in operation and the energy absorbed from direct and reflected sunlight. This heat is then conducted to variable conductance heat pipes mounted to external radiators at the upper end of the canister and radiated to space. The heat pipes are long narrow closed chambers with internal capillary wicking which provides pumping action. The wick is saturated with a volatile liquid (ammonia) in equilibrium with its vapour. Heat transport is established by applying heat at one end (the evaporator) and providing cooling at the other end (the condenser) with the heat being trasferred as latent heat of vaporization. The flow path is completed by capillary forces in the wick.

The variable conductance heat pipes are more complex than the fixed conductance type in that they contain a non-condensible gas (nitrogen) stored in a reservoir at the condenser end of each pipe. As the temperature of the evaporator end of the pipe falls a heating element raises the temperature of the reservoir, causing the gas to expand into the condenser, blocking the condenser region and effectively stopping heat pipe action. The length of condenser rendered inactive depends on the temperature level along the pipe. Conversely, with increasing evaporatorend temperature, the gas will recede into the reservoir making more active area of the radiators available for heat rejection to space. The signal for activating the reservoir heaters is supplied through a feedback loop consisting of a temperature control sensor and either a hardwire proportional controller or a computerdriven controller. The sensors are attached to the canister side walls or on simulated instruments located in two different zones separated by an insulating barrier. The simulators are either radiatively or conductively coupled to the canister walls.

During the mission, it is planned to: (1) operate the canister over set points (5-25°C) located on the walls and on the simulators themselves; (2) change the internal dissipation (in the simulators); and (3) demonstrate control in maintaining the two zones at differing temperatures. The system can be operated by a proportional controller maintaining a specific temperature at one sensor, or by a microprocessor that uses all available data to maintain the overall temperature of the canister at some level, in balance with the environment, irrespective of the preselected set point.

Contamination monitoring

Payloads operating in the bay of the Orbiter will be exposed to a variable gaseous environment. In addition to outgassing of the vehicle and the payloads themselves, the operation of altitude control systems, the venting of relief valves and the dumping of water for thermal control of the vehicle all represent molecular sources that may affect sensitive instrumentation, particularly equipment at cryogenic temperatures. Measurements have been planned for the series of four orbital test flights using the Induced **Environment Contamination Monitor** provided by the Marshall Space Flight Center and located, on this flight, behind the OSS-1 pallet in the Orbiter bay. Particulate measurements will be provided by the Shuttle/Spacelab Induced Atmosphere Experiment. A molecular contamination monitor on the OSS-1 pallet provides information on molecular species around the OSS-1 instruments, supplementing measurements from the Induced Environment Contamination Monitor.

The Contamination Monitor Package, sponsored by the United States Air Force, contains four temperature-controlled Quartz Crystal Microbalances which measure the accreted mass of molecular fluxes. These microbalances are identical to those contained in the Induced **Environment Contamination Monitor but** their operation can be monitored and the data analysed during the flight. Their temperatures may be reset by command after initial results have been analysed to optimize the operation of the sensors.

The instrument will monitor the mass accretion of condensible volatile materials during ascent, on-orbit operations and descent. After the mission, the data on mass build-up will be correlated with payload activities, Orbiter operational events, performance of other OSS-1 instruments and Induced Environment Contamination Monitor results. The activation energy of the major species of the accreted materials can also be estimated.

While minimal impact is expected for most instruments that may fly on Shuttle, long-term exposures of particularly sensitive optical components or cryogenic surfaces to the Orbiter environment may require special precautions.

Future prospects

The OSS-1 instruments will point the way to future developments in the use of the Shuttle for space science investigations. Two of the OSS-1 instruments will fly again on the Spacelab-2 mission: the Plasma Diagnostics Package will be released from the Remote Manipulator System to become a subsatellite and make measurements of the spacecraft wake at distances up to 20 km behind the Orbiter, while the Solar Ultraviolet Irradiance Monitor will be mounted on a solar pointer to continue UV irradiance measurements through the solar cycle. The data from other experiments will be used to design large UV and IR telescopes and other instruments for future flights in the Orbiter. Ultimately, the Shuttle program will include disciplinededicated flights and the development of space platforms serviced by the Shuttle.

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NEWS AND VIEWS

How life began

from Preston Cloud

THE prebiotic Earth, the beginnings of life and the evolution of biological complexity were the subjects of a small symposium* of 27 participants from Australia, Canada, the Peoples' Republic of China and the United States held recently at Jindabyne, New South Wales.

The now-large group of polyatomic organic molecules known from galactic space as a result of radioastronomy linked with spectroscopy extends the plausible choices available for the initial building blocks of life. Brown (Monash University) described contributions from (1) chemical evolution in the dark nebulae of the Galaxy; (2) chemical evolution in the presolar nebula followed by the arrival on Earth of its products in planetesimal debris; and (3) chemical evolution in Earth's primitive atmosphere and hydrosphere. The arrival in the early solar nebula of cold organic molecules from the interstellar stockpile appealed to Chang (NASA); had they been warmed on entry, ices would have melted, and a lot of aqueous chemical evolution might have occurred in restricted environments.

The heavily cratered surfaces of the solid planets and satellites were cited by Taylor (ANU) as evidence that planetesimal infall continued until about 3.8 Gyr (3.8×10^9 yr) ago, wiping out earlier records of earth history. Meanwhile Earth's core and mantle had formed, a conclusion drawn by Sun (CSIRO) from the constancy from 3.8 Gyr ago until the present of the ratio of P_2O_5 to TiO_2 in mantle-derived basalts. The motions of a fluid core can generate the magnetosphere that shields Earth's surface from the potentially lethal solar wind.

With a source of prebiotic organic molecules and such shielding, chemical evolution along the continuum from nonliving to living could occur at sites shielded from lethal UV wavelengths. The central problems of biopoesis (N.W. Pirie's word for the origin of life) are how to achieve the necessary concentrations of organic macromolecules, how to catalyse the essential reactions and how to accomplish chirality.

The chemistry of the atmosphere under

*The symposium was held from 13 to 15 December 1981 under the joint sponsorship of the Australian Academy of Science and the National Academy of Sciences of the USA.

which life arose is important. Earlier views invoking highly reducing conditions are giving way to geochemical evidence for a nearly neutral atmosphere. Fluid inclusions in 3.5 Gyr old barite show CO₂, H₂O, H₂S and possible hydrocarbons (Groves, UWA). An atmosphere at this time of CO₂, H₂O, N₂ and CO, with minor proportions of NH₃, H, CH₄ and H₂S, seems likely. Experiments on chemical evolution show small but finite yields of interesting organic molecules under such an atmosphere in the absence of free O₂. Carbonaceous chondrites might even more successfully concentrate amino acids and nucleotide bases in wet clay banks that could promote dehydration condensations. And the chemistry of Archaean sediments argues for very low and evanescent free O2. In fact, the real problem with oxygen is not to account for its absence in earlier times but for its presence later. Because the co-products of the processes that produce it are all thermodynamically unstable, free O₅ can accumulate only as the product of a kinetic

Sedimentary rocks 3.8 Gyr old are evidence for a substantial atmosphere and hydrosphere in earliest Archaean time. Despite the faint early sun of solar physicists, liquid H₂O has been present at Earth's surface from then onwards. McCulloch (ANU) described these oldest known sediments as implying derivation from undifferentiated source rocks—hence from crust formed no more than 200–300 million years earlier, about 4–4.1 aeons ago.

Returning to the question of biopoesis, the origin of replication was the subject of spirited discussion. Reanney (La Trobe University) proposed that RNA came first, as implied by the appearance of ribose before deoxyribose in existing biosynthetic pathways. But Langridge (CSIRO) reasoned that DNA came first because its bonds resist hydrolysis more strongly than those of RNA. Or did something else start the process? Burgess (Ludwig Institute) proposed that sugars were the logical beginners because they form readily, have a limited rotational freedom, will form helices with catalytic properties, are stable and link with lipids to form micelles.

But, in prebiotic evolution, how are sequences replicated and copying errors limited? Replication would be favoured, Reanney supposed, by day-to-night temperature variations. Experimentally, strands of DNA separate on heating and recombine when cooled. Such a mechanism could be important on an early Earth with shorter days and hence more rapidly repeated cycles.

Chirality may not be as much of a problem as once supposed. Brown remarked that once as many as five amino acids of the same handedness are strung together, they will preferentially add other amino acids of similar handedness from racemic mixes. To get five amino acids or sugars of the same handedness lined up by chance is entirely probable. And given a metabolizing, self-replicating structure, mutation is the next essential property. Copying error prescribes mutation and mutation predisposes to evolution.

Anderson (CSIRO) then showed how colloidal systems, bilayer vesicles and, ultimately, cells could result from the propensity for self-organization of amphipathic lipids and proteins. The distinctive properties of membranes permit the energy transduction that results in living cells.

Much attention at the symposion was directed to the earliest known forms of life and to their probable biochemical evolution. Evolution beyond fermentation requires photosynthesis and phosphorylation to trap and maintain a freeenergy flow. By what routes, Anderson (CSIRO) asked, could photosynthesis and respiration arise? Mauzerall (Rockefeller University) sees biosynthetic pathways as recapitulating evolution. Each porphyrin on the path to chlorophyll biosynthesis had a specific function in its own time. The first porphyrins of the haem/chlorophyll biosynthetic pathway, the uroporphyrins, were presumably the initial photo-oxidants that later became coupled to phosphorylation and photosynthesis. Bacteriorhodopsin, present in halophilic bacteria, is the most primitive surviving

Preston Cloud is at the Baas-Becking Geobiological Laboratory, Box 378, Canberra City, ACT, Australia. molecular device to promote ion flow across membranes.

Walter (Baas Becking Laboratory) reviewed the fossil evidence and Hayes the biogeochemical evidence for early evolution. They concluded that life was present by 3.5 and photosynthesis by 2.8 Gyr ago in the Warrawoona and Fortescue strata of Western Australia.

An extended discussion followed about the importance of sulphur in living systems, the geological record of sedimentary sulphate and S isotopes, the possible importance of methanogens and the question of when free O, from photosynthesis first became an important atmospheric gas. Groves noted that sulphates are uncommon in Archaean rocks whereas sulphides are abundant. Donnelly added that the sulphur isotope record of stratabound volcanogenic sulphide deposits calls for very low concentrations of oxidized sulphur in the Archaean hydrosphere. The record observed is consistent with the convergence of three important and perhaps related events ~ 2 Gyr ago: (1) the beginning of free O₂ as a permanent component of the atmosphere, (2) the end of methanogenesis as an important function of the biomass, and (3) the onset of dissimilatory sulphate reduction.

The emergence of the eukaryotic cell at some time after ~ 2 Gyr ago was the focus of the final session at Jindabyne. Walter reviewed the biogeological evidence for the rise of O, during Earth history, the probable time of emergence of the eukaryotes and alternative hypotheses for eukaryotic origins. Runnegar (New England University) observed that even anaerobic eukaryotes need O2 for the production of collagen and components of the cell wall. Thus eukaryotes probably made their first appearance later than 2 Gyr ago. But how? Langridge observed that differences between organellar and nuclear DNA of eukaryotes could result either by derivation of the organellar DNA from the nucleus by a transfer analogous to that involved in the origin of some viruses, or by endosymbiosis.

Lastly the conference turned to the origin of membrane-bound organelles, considered distinctive of the eukaryotic cell. Langridge noted that cytochrome c amino acid sequences and 5S rRNA nucleotide sequences imply a date of ~ 1.8 Gyr ago for the divergence of the eukaryotic from the prokaryotic level of organization - a date consistent with biogeological evidence. No serious alternative was offered to serial endosymbiosis for the origin of the eukaryotic complement of membranebound organelles. Discussion concentrated instead on which of these organelles might have come first. Starting with a nucleated host, most preferred first to add mitochondria of probable bacterial origin and then chloroplasts, whose 16S rRNA sequences imply a blue-green algal origin.

Was Galileo 2,000 years too late?

from David W. Hughes

SIDEREUS NUNCIUS was published in Venice in 1610 and revealed to the renaissance world "great, unusual, and remarkable spectacles as observed by Galileo Galilei, Gentleman of Florence, Professor of Mathematics in the University of Padua, with the aid of a spyglass". The title page also announced that the greatest discovery was that of the "four planets swiftly revolving about Jupiter at differing distances and periods, and known to noone before the Author recently perceived them and decided that they should be named The Medician Stars". One of Galileo's notebooks records how Jupiter was seen on 7 January 1610 with three companion 'fixed stars', two to the east and one to the west. Galileo concluded, after a week of observation, that "there are three wandering stars around Jupiter, previously invisible to everyone".

Galileo's claim to priority has recently been shown to be unjustified; and it is not a matter of being just pipped at the post, but of some 2,000 years.

Xi Ze-zong, of the Institute for the History of Natural Sciences, Academia Sinica, has recently reported in *Chinese Astronomy and Astrophysics* (5, 242; 1981) that Gan De observed Ganymede in the summer of 365 BC.

Gan De was one of the earliest Chinese astronomers and was an assiduous observer of the heavens, and in particular of the planet Jupiter. He wrote two books, Treatise on Jupiter and Astrological Prognostications. Both have been lost but fortunately portions have been preserved in The Kaiyuan Treatise on Astrology which was compiled by Qutan Xida between AD 718 and 726. Gan De is quoted as having said:

"In the year of chan yan . . ., Jupiter was in Zi, it rose in the morning and went under in the evening together with the lunar mansions Xunü, Xü and Wei. It was very large and bright. Apparently, there was a small reddish star appended to its side. This is called 'an alliance'."

The reference to the zodiacal division Zi and the lunar mansions reveals that the observation was made in the summer of 365 BC and the use of the term 'in alliance' that the small star is described as a subsidiary of Jupiter. The colour 'chi' is a light red.

Xi Ze-zong puts forward no explanation as to why this reference to a satellite of Jupiter has been overlooked until now.

Which satellite was it? Jupiter has four 'galilean' satellites, Io, Europa, Ganymede and Callisto, and when Jupiter is in opposition, at its closest to Earth, these have magnitudes of 4.9, 5.3, 4.6 and 5.6 respectively (a change of 1.0 in magnitude is equivalent to a factor of 2.5 in brightness and bright objects have low magnitude values). At opposition the maximum

angular separations between the satellites and Jupiter are 2.3, 3.7, 5.9 and 10.1 arc min respectively. Ganymede is thus the brightest moon and can be as much as 5.9 arc min away from Jupiter — one-sixth the apparent diameter of the Moon. Unfortunately, Jupiter then has a magnitude of around -2.6 and is thus 760 times brighter.

Xi Ze-zong used a planetarium to simulate two sources differing in brightness by a factor of 760 and separated by 5.9 arc min. People with good eyesight could detect the fainter object. Xi Ze-zong could, however, have saved himself some trouble by turning to a more recent 'ancient tome'. Admiral William H. Smyth wrote in his Celestial Cycle, Vol. 1 (1844) that

"Certain esprits fort express surpise that Galileo should have been so gratified by this discovery since they hold that the satellites of Jupiter are often seen with the naked eye and they cite the Apennines and Etna and the West Indies and various other fine-climate places as the spots where such a feat is frequently done."

Smyth concluded, however, that the observer must not only be at a site of fine seeing and excellent atmospheric clarity but must also possess "visual organs of extraordinary power".

Two problems still exist. We cannot be sure whether Gan De saw Ganymede or Callisto. Ganymede is the more probable simply because it is the brighter. The reference to the 'light red' colour is also mystifying. Ganymede is too faint for its colour to be perceived with the naked eye. Even in a telescope it appears white and has a very similar colour to that of Jupiter.



This the hundredth article written written for News and Views by David W. Hughes, a lecturer in physics and astronomy at the University of Sheffield. The first, 'Meteors and Meteorology', was published on 13 June 1970 (Nature 226, 1008).

Molecular recognition and the future of monoclonal antibodies

from David Lane and Hilary Koprowski

A MOST striking revelation to emerge from recent experiments with monoclonal antibodies has been the repeated demonstration of cross-reacting sites on molecules in which conventional serology had failed to detect homology. The most obviously suggestive of these crossreactions are those which have been found between the large T antigen of simian virus 40 (SV40) and normal proteins of host cells1,2. At least four monoclonal antibodies against the T antigen, which is strongly implicated in the oncogenicity of the virus, can be shown to cross-react very specifically with host proteins, one of which, a ubiquitous protein of molecular weight 68,000, is regulated by cell growth.

Other cross-reactions, all equally specific but not all as obviously suggestive, have been detected between various other more or less related molecules. The well characterized rat monoclonal antibody 42-21, for example, binds both Thy-1 antigen and the Vx chain of immunoglobulin molecules bearing the TEPC-15 idiotypic determinant3. A monoclonal antibody obtained after immunization of mice with herpes virus4 binds a glycoprotein specific to the virus and virus-infected cells, but also crossreacts with neutral glycolipids extracted from normal uninfected cells⁵. Yet other monoclonal antibodies cross-react with two cytoskeletal proteins, tropomyosin and vimentin6; and another cross-reacts with Thy-1 antigen and intermediate filaments⁷. Probably yet more apparently capricious cross-reactions have been detected but not reported.

What do they mean? The most exciting possibility is that these antibodies are detecting the conserved recognition sites underlying the specific macromolecular interactions that control most important biological processes. But there are serious difficulties in using monoclonal antibodies as the sole indicator of molecular identity. These difficulties are well known to immunochemists, but they may not be fully appreciated by the growing numbers of workers new to the field of immunochemistry who are now turning increasingly to monoclonal reagents in preference to conventional antisera. Before expanding on the potential of monoclonal antibodies in the exploration of molecular recognition, therefore, we shall reconsider the character of polyclonal antisera and the nature of antibody specificity, and discuss how these bear on

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the interpretation of immunological crossreactions between molecules.

There are two crucial points to be considered, the first of which is the heterogeneity of conventional polyclonal antisera. A conventional antiserum raised in a rabbit against bovine serum albumin, for example, contains a large number of different antibodies directed against different discrete determinants or epitopes on the surface of the bovine albumin molecule. Some of these epitopes will also be present on a related molecule such as human serum albumin, and the two antigens will thus be cross-reactive. Other epitopes, on the other hand, will be present only on one or other albumin molecule. By

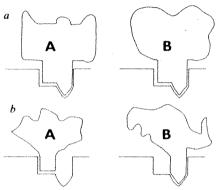


Fig. 1 Two ways a monoclonal antibody could cross-react. a, Protein antigens A and B have the same shape over part of their surface. b, A and B have different structures but interact with the same antibody molecular.

appropriate absorption experiments polyclonal antisera can be used to distinguish the cross-reactive and the noncross-reactive epitopes and are thus powerful tools for such purposes as determining phylogenetic relationships between species⁸. Evidently a single monoclonal antibody will never yield this kind of information; and so complex are conventional antibody responses that it would be extremely difficult to mimic them with artificial 'cocktails' of monoclonal antibodies.

But the ease with which homologies between proteins can be detected by conventional antisera depends not only on the intrinsic structure of the antigens but also on the host response to them, which can result in important blind spots in individual sera. For the antibodies produced in a normal immune response are not evenly directed against all parts of the surface structure of the antigen. Rather, most antigens have clearly immunodominant regions which in some cases, for example the influenza haemagglutinin, have been closely mapped⁹. The relative immunodominance of given regions on a protein molecule may

vary between different host animals. For example, different strains of inbred mouse preferentially make antibodies to different areas of the staphylococcal nuclease antigen¹⁰; and certain mutant determinants on human haemoglobin molecules cannot be recognized by rabbit antisera because they cross-react with rabbit haemoglobin, to which the animal is immunologically tolerant¹¹. Such factors will restrict both the ability of individual conventional sera to detect cross-reactions between antigens and, of necessity, the range of monoclonal antibody specificities that can be obtained from a particular cell fusion.

The second important consideration in the interpretation of cross-reactions is directly relevant to the use of monoclonal antibodies, and concerns the nature of the antibody response to a single epitope and the interactions between one pure clonal antibody and that structure. In a conventional antiserum, not only are numerous different antibodies directed against different determinants on the same molecule, but a very large number of different antibodies will react with each single epitope, recognizing it with different degrees of affinity. This extraordinarily diverse response to a single epitope, which may be a very simple artificial determinant such as dinitrophenol, has attracted detailed examination because of its implications for the genetic basis of antibody diversity. A theoretical consideration of the problem led Talmage¹² to propose that one antibody binding site might be polyfunctional and accommodate more than one epitope. He then reasoned that some antibodies would be produced in the immune response to several different antigens and the full range of specificities might be achieved by fewer different antibodies than might at first seem necessary. Thus epitope A would stimulate clones of antibodies with specificity for epitopes A, B, C, D; A, E, F, G; A, H, I, J, and so on. The serum would then bind predominantly epitope A, present on the eliciting antigen. The important implication for monoclonal antibodies is that one would not expect them to show the same degree of A-dominance as the polyclonal serum: they might react equally well with another epitope, for example B, that the whole serum recognizes at such a low titre compared with A as to render it undetectable. This important hypothesis was examined in detail by Richards and colleagues13 who obtained direct experimental proof for the existence of polyfunctional binding sites; thus, a myeloma protein (460) was found to bind two structurally unrelated molecules dinitrophenol-lysine and 2-methyl-1, 4-naphthoquinone (menadione) at sites that could be shown to be physically

Bounded brainpower? from Paul Davies

Is there a limit, due to fundamental physics, on the rate at which information can be processed in a computer? "Yes" claims Jacob Bekenstein, the co-inventor of the quantum black hole. In a provocative paper published in *Physical Review* a year ago, he claimed that the bread and butter of information transfer—energy and entropy—cannot be varied at will, but are constrained by the bound

$$\frac{\text{entropy}}{\text{energy}} < \frac{k}{\hbar c} \times \text{size} \tag{1}$$

where $h = h/2\pi$, the size of the system is defined in some suitable all-embracing sense, and k, h and c are respectively Boltzmann's constant, Planck's constant and the velocity of light.

Bekenstein went on to assert (*Nature* **292**, 112; 1981) that this bounded ratio of entropy to energy also constrains the rate of information processing to be less than

$$\frac{\pi k}{\hbar}$$
 × energy (2)

Taking into account the need to flush out the heat produced in a hypothetical computer by the information's energy, Bekenstein estimated the upper limit to be about 10¹⁵ bits per second (still pretty fast).

Now all this has been called into question by David Deutsch of Oxford University's Department of Astrophysics. In *Physical Review Letters* (48, 287; 1982), Deutsch challenges the fundamental basis of Bekenstein's analysis — the existence of an entropy to energy bound [equation (1)]. Energy, he points out, is not itself a measurable quantity in non-gravitational physics. Only energy differences are relevant to devices such as computers. Bekenstein's formula (1) might work for systems such as black holes, where gravity plays a part, but it has no business to interfere where

gravity is unimportant. (Energy, having mass, is a source of gravity, so its absolute value can be measured gravitationally.)

Deutsch reworks Bekenstein's calculation for energy differences and finds that there is no upper bound on the entropy to energy difference ratio. He then argues that it is this ratio that is relevant to information processing. The essential point is that not all the energy carried by the information generates heat in the computer, only the energy change due to the encoding procedure. The rest is invisible to everything except the gravitational field. He also throws in, for good measure, the retort that, in any case, it is in principle possible, even in quantum physics, to retrieve information by measurements that leave the information undisturbed, and hence do not produce heat.

So the current state of play is that Bekenstein's bound (1), while not incorporating the newtonian gravitational constant G explicitly, is nevertheless a gravity formula in disguise, to be used only when the system concerned runs on gravity power. In that case, if a computer is too big, its thinking time is limited by the speed of light; too small and it implodes into its own black hole. In this sense, bound (1) may provide a genuine limit, which Deutsch estimates at around 10^{42} bits per second!

Alas, even the gravitational applications of equation (1) have recently been called into question by William Unruh, Robert Wald, Don Page and Stephen Unwin in some lively exchanges (*Physical Review* D, in the press). Evidently the embattled Bekenstein has caught the attention of more than the computer industry.

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discrete (though the two haptens did compete for the binding site).

In light of these considerations, there are two distinct explanations of the physical basis of monoclonal cross-reactions. One interpretation is illustrated in Fig. 1a, where protein antigens A and B share a small and precise detail of their surface topology. Such a determinant might not normally be detected using conventional antisera in which most of the antibodies will be directed against other A-specific or B-specific structures; only dissection of the antibody response using the monoclonal technique reveals the shared structure. The question then becomes one of just how significant such small homologies are.

In the second explanation (Fig. 1b), the two protein antigens have dissimilar structures but interact with the same antibody molecule. It is notable that the

two different antigens could still compete for binding to the antibody because of steric constraints, even though their reactive epitopes, by analogy with the myeloma 460 example, occupy discrete sites on the molecule. However, the affinity of the antibody for antigens A and B would then be expected to be much lower than in the alternative model illustrated in Fig. 1a. In the second model (Fig. 1b) there is no reason to assume any biological relationship between the cross-reacting antigens; the only factor of interest in the system is the existence of the cross-reactive antibody itself. The production of such antibodies, for instance, could contribute to an autoimmune response induced by a structurally completely unrelated antigen. These two models represent the extreme alternatives and of course it must also be possible for the epitope on antigen A to be

only partly homologous to the epitope on antigen B, so that the antibody binds antigen A with higher affinity. Detection of such cross-reactions, whatever their molecular basis, means that reaction with a monoclonal antibody cannot itself be interpreted as proving molecular identity. For example, the reaction of a monoclonal antibody with two different cell types still requires biochemical verification that an identical molecule is being recognized in each cell. Similarly, such reactions might constrain the use of monoclonal antibodies in radioimmunoassay. Clearly, both of the extreme models have some validity, and examples of both types of cross-reaction will be discovered. Our bias is that the physical basis of the majority of crossreactions detected so far is nearer to that illustrated in Fig. 1a, because they seem so highly specific. For example, the Pillemer and Weissman antibody shows no detectable binding to Thy-1-negative mutant lymphoma cells or to any IgG except that of the T-15 idiotype, and one of the SV40 T monoclonals has a very high affinity for T yet uniquely recognizes a single low-abundance 68,000 host protein on Western blots of total proliferating cell homogenates. (It does not recognize any protein at all when the extracts are made from the same cells in a quiesent state.)

The question then becomes one of just how functionally significant these small homologies between proteins are. That some are significant is nicely illustrated by the sweet-tasting proteins thaumatin and monellin. Hough and Edwardson14 have shown that polyclonal antibodies against thaumatin mimic the sweetness receptor, in that other sweet-tasting substances displace 125 I-labelled thaumatin from the antibody with an efficiency that correlates well with their relative sweetness. Of particular interest is the very effective competition between thaumatin and another very sweet-tasting plant protein, monellin, because the primary sequences of these two proteins show surprisingly little homology. Thaumatin has a single chain of 207 amino acids, and monellin has two chains totalling 94 amino acids; the homology is limited to five identical tripeptides15. We can infer that the antibody response has been selective for the biologically active region of the molecule, and by concentrating itself in a biologically relevant structure has mimicked a receptor.

A similar example of receptor mimicry by antibodies can be seen in the studies of Sege and Peterson¹⁶ where anti-idiotypic antibodies, raised against antibodies to insulin, were found to possess insulin-like activity themselves in biological tests. Cross-reactive monoclonal antibodies may therefore lead us to discover biologically important relationships between proteins and other macromolecules that could not have been detected by other means. After all, biological macromolecular interactions are all about three-dimensional shape

recognition, which is what antibodies do.

The virus-host interface is an especially interesting area in which one might expect to find such homologies. In the case of the oncogenic RNA viruses, Blair et al. 17 have shown the existence in normal mouse DNA of a cellular homologue to Moloney murine sarcoma virus and have demonstrated transformation of murine cells by recombination of the cloned homologous sequences and plasmids containing proviral long terminal repeat sequences of Moloney sarcoma virus. Derivation of src sequences of two murine sarcoma viruses, Harvey and Kirsten, from normal genes (sarc) of different vertebrate species such as rat, chicken, human and mouse has also been described recently¹⁸. These sequence homologies between retroviral onc genes and cellular genes have not been detected in analogous experiments using the transforming genes of the DNA tumour viruses. Structural homologies between these viruses transforming gene products and host proteins must exist19, however, and they may now be detected by monoclonal antibodies.

It is our hope that many exciting new correlations between important macromolecules not readily detectable by other means will emerge from these studies and increase our understanding of biological organization.

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Genetic diagnosis of the fetus

from Stuart H. Orkin

THE past decade has seen rapid progress in the development of reliable methods for the identification of genetic disorders in the human fetus. Chromosome abnormalities, such as Down's syndrome, are now routinely detected during mid-trimester by the analysis of fetal cells shed into the amniotic fluid. Structural abnormalities. such as neural tube defects, may be due to many factors but can now often be detected by screening for α -fetoprotein in maternal blood and amniotic fluid. Enzyme deficiencies related to single gene disorders may also be identified from the culture of fetal cells, obtained by the safe and simple aspiration of amniotic fluid in midtrimester.

Many disorders, however, are not readily detectable in fetal cells from amniotic fluid because expression of the relevant genes is restricted to particular cell types. Conditions affecting either the structure or synthesis of the polypeptide chains of haemoglobin are the best known examples. Sickle cell anaemia, the result of the most common structural abnormality of the human β -globin chain, and β -thalassaemia, the consequence of underproduction of β -globin chains, are disabling genetic disorders of widespread occurrence. Only since the mid-1970's have new methods, the most recent of them employing the techniques of molecular

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biology to analyse the genetic material itself, begun to show success in the early diagnosis of these diseases.

The search for suitable diagnostic methods first focused on acquiring fetal blood samples since the β -globin gene is expressed, although at low-levels, during mid-trimester. This approach depended not only on reliable chromatographic methods for assaying the globin products, but also on the development of relatively safe techniques for sampling fetal blood. Direct aspiration of placental veins via a fetoscope has been especially successful although blind aspiration of the placenta has been used as well, with good results1. In both cases the risk of sampling to the fetus is of the order of five per cent, considerably above that of simple amniotic fluid taps. Such a risk is certainly acceptable in families faced with a one in four chance of having a severely affected child. By virtue of the pioneering work of Nathan, Kan, Kazazian, and their colleagues, the prenatal detection of haemoglobinopathies using fetal blood is now an accepted procedure and has been used in more than 1,800 cases2. This, in conjunction with genetic screening and counselling has reduced the birth rate of β -thalassaemics in several regions. As has often been the case in work of this kind, the technical developments have brought additional benefits it is now possible to detect haemophilia and rare granulocyte dysfunction syndromes in utero, and to assess directly fetal cell

chromosomes where amniotic cell analysis may suffer from contamination with maternal cells

The advantages of determining the presence or absence of a genetic disorder by direct gene analysis, as well as the risks involved in the acquisition of fetal blood, led in 1978 to efforts to apply novel techniques of DNA analysis to prenatal diagnosis3. Some forms of thalassaemia were known from molecular hybridization experiments^{4,5} to be due to structural gene deletions. By using specific DNA probes for globin sequences that could be labelled to high specific activity^{6,7}, bacterial restriction enzymes that cleave DNA at specific nucleotide sequences8 and the simple gel method devised by Southern9 it was possible to detect such deletions in a DNA sample of 10 µg or less. This amount of DNA could be obtained from fetal cells either directly on withdrawal of amniotic fluid or after in vitro culture. However. although the ability to detect these rare deletion states was a step forwards, for both sickle cell anaemia, which is due to a substitution in codon six of an normal β -chain sequence, and β -thalassaemia, the result of many different single nucleotide substitutions either in the coding or intervening sequences of a normal β -gene¹⁰, a more specific assay was required.

A remarkable finding by Kan and Dozy presented new alternatives. They identified a DNA polymorphism (for the enzyme Hpal) in the 3'-flanking region of the β -gene that was quite often linked with the sickle β -gene itself¹¹. By examining the B-gene fragments after Hpal digestion in family members, diagnosis of sickle cell anaemia was possible in many, though unfortunately not all, families known by linkage analysis to be at risk¹². Limitation of linkage between the polymorphism and the sickle gene to about 60-70 per cent and its variation in different world populations ultimately restricts the usefulness of this approach. The finding of additional restriction polymorphisms in the β -gene cluster does, however, extend its applicability to sickle cell diagnosis 13. Similarly, β -thalassaemia may often be diagnosed now by linkage analysis. 14,15.

Nevertheless, direct detection of the primary gene mutation would be desirable as linkage analysis generally requires careful family studies with several restriction enzymes, an affected family member and often considerable amounts of precious fetal DNA. The power of restriction enzyme mapping is such that a single base change in a restriction enzyme recognition site can be readily detected. Nienhuis first noted that the restriction enzyme MnII (recognition site GAGG) would cleave the normal but not the sickle cell codon six16. However, because Mn/I cleaves β -genes DNA very frequently, experimental use of this enzyme for sickle cell detection has not been successful.

Recently, Geever et al.17 noted that a different restriction enzyme Ddel (recognition site CTNAG) would cleave normal, but not sickle, DNA at the sixth codon. Knowing the sequences of the normal β -gene and its immediate flanking region, the sizes of the restriction fragments expected from the normal and sickle cell DNAs could be predicted. Using electrophoresis of DNA fragments in acrylamide rather than the usual agarose, covalent transfer of fragments to a solid support and larger amounts of DNA (20 µg or more). Geever et al. could distinguish the B from the normal gene in total DNA. With a similar assay Chang and Kan18 showed that the approach could be applied to prenatal diagnosis. The detection of the β^{S} in these studies is an experimental tour de force given the relatively small size of the DNA fragments detected (less than 0.4 kb). In view of the technical difficulties of this approach Chang and Kan have advocated its use when the HpaI polymorphism is not informative.

Even this achievement may now be superceded - another restriction enzyme has been discovered that cleaves a subset of *DdeI* sites. While it cuts normal β -gene at codon six it does not cleave Ddel sites just upstream from the gene. The size of the DNA fragments from normal and sickle DNA should be considerably larger than those obtained using DdeI and more suitable for gene mapping. These predictions have recently been confirmed in the present author's laboratory¹⁹. The β ^S gene can now be detected directly following digestion with this new enzyme with a sensitivity comparable to detection of the HpaI polymorphism in the usual Southern gene mapping analysis. We believe that this constitutes a simple and reliable assay for the sickle mutation in DNA.

The prospects for direct detection of B-thalassaemia mutations are less favourable, however. Some β-thalassaemia mutations can be detected directly by the alteration of the β -gene map by a specific substitution, but most, at least among Mediterraneans, cannot. Linkage aĥalysis remains, however, a useful technique in these situations^{10,14,15}. Other single-gene disorders may, in principle, be identified by linkage analysis using human DNA fragments cloned from various chromosomes, thereby extending this approach to other conditions.

Families seeking prenatal detection of genetic disorders generally choose termination of pregnancy if the fetus is found to be affected with the disease for which it is at risk. The trauma for the family, both physical and psychological, is great, especially as diagnosis is not established until the latter portion of the second trimester. Earlier diagnosis would be very desirable if risks to the fetus or mother were not appreciable. Niazzi et al.20 and Kazy et al. 21 have reported trophoblast or chorion biopsy by the transcervical route at 6-12 weeks gestation. Such material provides fetal cells, that can be grown in vitro if necessary, suitable for

chromosome, enzyme and DNA analyses22. Although early study has shown no complications associated with the prodecure, more investigation will be needed before it gains wide acceptance. Nevertheless, the prospect of obtaining fetal cells in the first trimester is exciting indeed. It will obviate the "race to the wire" that sometimes prevails in midtrimester diagnoses and may ultimately alleviate considerable psychological and physical morbidity. The availability of such procedures will provide further incentive to find methods for DNA analysis, either direct or by linkage, for many genetic diseases.

Finally, we may wonder whether totally noninvasive approaches to diagnosis of disease in the fetus might not be possible. Fetal cells normally pass in small numbers into the maternal circulation. Specific enrichment of fetal cells by cell sorting, perhaps using recently developed monoclonal cell surface-specific antibodies23, may eventually allow fetal diseases to be detected in cells obtained from maternal blood. If this is likely to be useful in situations where DNA is analysed, it may be necessary to design novel gene

probes that extend the sensitivity of present gene mapping methods. At the current pace of progress in this field, we can anticipate that we will soon be surprised by as yet unforseen developments.

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Trace elements and ocean chemistry

from Michael L. Bender

THE article in this issue of Nature by Elderfield and Greaves (p.214) reflects two important trends in the study of the marine chemistry of the trace metals. First, there is increasing interest in the detailed chemical behavior of trace elements in the oceans the complexing of metals with anions and organic ligands, particle adsorption and the occurrence and the implications of trace metal redox reactions. Second, we can see the trend towards application of four of the sacred instruments of hard rock geochemistry - lead, strontium and neodymium isotopes and rare earth element distributions — to problems in marine geochemistry.

The earliest successful research on marine trace metals, begun in the early 1960s, concentrated on elements whose distribution in seawater could be accurately measured by the radiochemical study of uranium and its daughter Ra²²⁶. Subsequently, the approach has been extended to include the less abundant radioisotopes of thorium, radium, protactinium, polonium and lead. In the mid-1970's, a number of laboratories began to study the transition metals manganese, iron, nickel, copper, cadmium, zinc and chromium, which are of great interest because they are both essential and toxic to organisms, and

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because they are enriched in both hydrothermal and normal deep sea sediments. During the last few years, considerable attention has been paid to the metalloids tin, selenium and germanium, and the methylated compounds which they form. Finally, extensive effort has gone into determining the oceanic distributions of the global pollutants, lead and mercury.

Such studies have given us a broad understanding of marine trace element geochemistry. A number of elements, including thorium, protactinium, polonium, lead, iron and manganese, are highly reactive and are scavenged from seawater into sediments within a few decades. Others, including copper, nickel, cadmium, zinc, selenium, and cobalt, are rapidly removed from surface water by sinking tissue, but redissolved when the tissue is oxidized in the deep sea. They remain in the oceans for 103-105y before their final removal into the sediments. Selenium and chromium are present in the dissolved form in two oxidation states, while manganese and iron are rapidly scavenged once the reduced species are oxidized. The ocean's sources of trace elements - input from rivers, submarine hydrothermal processes (at least for manganese), and dissolution from aerosols (for manganese, lead, and copper among other elements) - are fairly well known. The removal mechanisms are less well understood, although it is clear that burial

of trace metals carried to the sediments by falling tissue is the dominant process.

Most of our understanding of the marine cycles of trace metals is based on concentration variations in seawater. Such data cannot, however, give us a profound understanding of the physical chemistry governing their behavior. To meet this problem, some groups have studied the inorganic ion pairing reactions of the trace metals, metal-organic interactions, and the reactions between trace metals and important inorganic and organic surfaceactive phases. Other groups are studying trace metal behavior by radiolabelling trace metals in enclosed ecosystems, and determining their uptake rates and solid phase distributions.

The rare earth elements (REE) have a unique role to play in extending our understanding of marine trace metal geochemistry. Their ionic radii, and hence ion pairing and surface adsorption stability constants, vary in a predictable way with atomic number. As a consequence the importance of complex formation and adsorption in the marine cycles may be inferred directly from the subtle interelement differences in REE distributions. While all REE exist in the +3 oxidation state, Ce and Eu also exist in the +4 and +2 oxidation states, respectively. By comparing their distributions with those of adjacent rare earths, it is possible to judge how their behavior depends on oxidation state. The relative abundances of rare earth elements are distinctly different in various continental source materials, seawater, and sediments and the abundance patterns can serve as 'fingerprints' that identify sources of the elements in seawater, sediments, and manganese nodules. The isotope composition of one of the REE's, neodymium, itself serves as a fingerprint, since it varies in different source rocks as a result of Sm radiodecay to Nd143. Finally, the power of all these tools for studying REE chemistry is enhanced, as Elderfield and Greaves show, by two happy coincidences. First, REE are affected by nearly all of the important processes influencing marine trace metal behavior: atmospheric input, adsorption and scavenging onto falling particles, coprecipitation, ion pairing, and redox reactions. Second, the scavenging residence time for REE - apparently about 400y - is shorter than the oceanic mixing time (1,600y), so that REE and Nd isotopes always reflect elemental sources. If the residence time were longer than the mixing time, the fingerprinting would be less distinct and the REE studies would be less useful.

The second trend reflected in Elderfield and Greaves article can be seen in a number of recent papers. Pb isotopes are now being used to trace the anthropogenic lead distribution, while Sr isotopes are being used to study low and high temperature seawater-basalt exchange as well as

carbonate diagenesis. Elderfield et al. (Geochim, cosmochim, Acta 45, 1231; 1981) used REE distributions to characterize the source of these elements in North Pacific nodules and sediments. Goldstein and O'Nions (Nature 292, 3241; 1981) have used the strontium and neodymium isotope compositions to show that, in manganese nodules, those elements have been precipitated from seawater, while Piepgras and Wasserburg (Earth planet, Sci. Lett. 50, 128; 1981) have shown that the isotope composition of dissolved neodymium is different in the Atlantic and Pacific oceans (indicating a residence time less than the mixing time).

Rare earth concentrations in seawater were first measured successfully in a few samples during the sixties by Goldberg et al. (J. geophys. Res. 68, 4209; 1963) and Høgdahl et al. (Adv. Chem. Ser. 73, 308; 1968.) Since then only a small amount of additional data has been published. The

paper by Elderfield and Greaves represents a major advance in the accuracy of measurement, and through the study of a complete water column profile allows an appreciation of the roles of particles in removing REE from the shallow waters of the ocean and remineralization redissolving them at depth.

The data allows Elderfield and Greaves to outline the main aspects of both the long term mass balance of REE in the oceans, and the pathways involved in the internal cycling of REE. The context is thus set for more detailed studies of the oceanic distributions of the REE. The results promise to give us a profound understanding of the mass balance, internal cycling, and physical chemistry of REE's in seawater, and thereby greatly extend our appreciation of how the fundamental chemical properties of trace metals influence their behavior in the marine environment.



100 years ago THE CHANNEL TUNNEL

The two schemes for a tunnel beneath the Channel, on the comparative merits of which a Parliamentary Committee will probably take evidence in the course of the year, are based, like those which have preceded them, chiefly on geological considerations. The Weald of Kent and the Bas-Boulonnais, once in all probability geographically continuous, still constitute a single geological area.

The two schemes which are offered for tunnelling through the Chalk may be briefly stated as follows:—

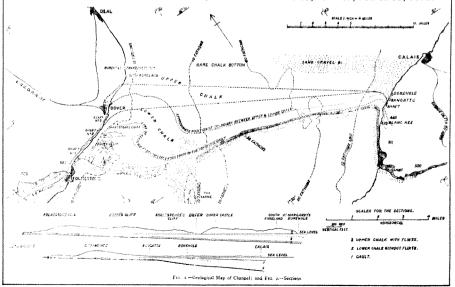
1. The Channel Tunnel Company, with which Sir John Hawkshaw is connected, propose a tunnel starting from Biggin Street, Dover, with a gradient of 1 in 80, passing under the north spur of Dover Castle Hill, and thence continuing to a point on the shore known as the Fan Hole at a distance of 2 miles, 4

furlongs, 2150 chains from Biggin Street, and at a depth of 115 feet below high water ordinary spring tides. From this point it will run across the straits to join the French tunnel, which commences near Sangatte, and as Sir J. Hawkshaw has always advocated a straight line of tunnel, we presume that such will be the case here.

2. The Submarine Continental Railway Company, with which Sir E. Watkin is associated, propose a tunnel connected with the South-Eastern Railway, about two furlongs west of Folkestone entrance of the Abbotscliff Tunnel by a tunnel descending at a gradient of 1 in 52 to the bottom of the No.2 Shaft, near the west end of the Shakespeare Tunnel, at a depth of 126 feet below high water ordinary spring tides. From this point the tunnel will continue for about a mile towards the head of the pier in a direction slightly diverging from the shore, and finally curving round, will fall into the line of the French tunnel near Sangatte.

So far as our information goes at present it seems that the most serious obstacle will be water, and it is therefore on their relative liability to flooding that the proposed tunnels must be judged.

From Nature 25, 464-465; March 16, 1882.



REVIEW ARTICLE

The map sense of pigeons

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Homing pigeons are able to return to their lofts after displacement to unfamiliar sites hundreds of kilometres away. A map sense enables them to determine their location, and a hierarchy of compass senses orient their flight. Recent experiments implicate magnetic and olfactory cues as the basis of the mysterious map sense.

HOMING pigeons can be taken from their lofts and transported hundreds of kilometres in covered cages to unfamiliar sites and yet, when released, be able to choose fairly accurate homeward bearings within a minute and fly home. From direct tracking we know that a homing pigeon typically circles near the release site once or twice, edging in the general direction of home, and then straightens out and flies off at nearly the correct bearing1 (Fig. 1a). At most sites the usual vanishing bearing—the direction in which the bird is lost to view or, if it is carrying a radio transmitter, in which its signal fades out-is reliably left or right of the true home bearing. This consistent deviation is known as 'release-site bias' 2,3 since it varies from site to site (see Fig. 1b) or as 'preferred direction' 4,5 since it seems to impose a fairly consistent directional tendency onto the homeward direction of birds from any one loft (NNW in Fig. 1b). However, the exact route home is quite variable, and even the same bird released time and time again from the same site never seems to fly the same route twice. Once near home pigeons rely on visual landmarks to reach the loft⁶. Detailed vision is not necessary until the very last stages of the homing process, however, since birds wearing frosted lenses can get to within a few kilometres of home⁷ (Fig. 1c). Moreover, pigeons can even be trained (though with great difficulty and high attrition) to home at night8. Pigeons are not unique in their ability to locate precise targets from great distances—animals as diverse as monarch butterflies and sea turtles perform similar feats as part of their normal migratory repertoire—but the willingness of the pigeon to home on demand, day after day, has made it the favoured vertebrate for navigational research.

Aside from reading the minds of the experimenters (a possibility which has not escaped investigation), there are only three basic explanations for the remarkable ability of pigeons to home⁹: (1) the birds might keep track of their outward displacement (the system of many short-range species such as honey bees); (2) they might follow some home-centred gradient to their goal; or (3) they might have a true map sense which would permit them to 'place' themselves with respect to home on some internalized coordinate system.

For pigeons (and probably most other wide-ranging animals) the first two alternatives seem unlikely. If pigeons had to measure their displacement (presumably by keeping track of the direction and degree of acceleration and deceleration of the various turns, and timing the individual legs of the journey), simply transporting them in the dark or with constant rotations ought to impair or eliminate their ability to orient. This treatment, however, has no effect¹⁰⁻¹², nor does transport under complete anesthesia^{10,13,14}, or bisection or surgical removal of the vestibular apparatus¹⁵⁻¹⁹. Another model for an inertial system might involve a magnetic compass to measure the direction of each leg of a journey, and a time sense to estimate the length of each part. Birds transported to the release site wearing magnets or otherwise subjected to an artificial magnetic field, however, are often not affected (refs 8, 11, 12; but see below). Unfortunately, no one has yet performed the crucial experiment of transporting pigeons in total darkness anesthetized, rotating, and with the field reversed all at the same time.

The gradient alternative is also entirely lacking in experimental support. Such a strategy, based as it is on a 'getting warmer' system of movement, requires no compass sense. Yet pigeons have been shown to have compasses and to depend on them for homing; as we shall see, birds may be made to fly directly away from home by such relatively simple manipulations of their compass sense as clock shifts.

The compass sense

The idea that pigeons must have both map and compass was first put forward by Kramer²⁰. As he pointed out, an animal transported to an unfamiliar site probably needs both: a map sense to determine the direction of displacement from home, and a compass to find that direction. (In theory, of course, a pigeon could home by means of successive map readings without any recourse to a compass; but the clock shifts described below, as well as the ability of the birds to select a reasonably accurate homeward bearing within 100-500 m of the release site, render this possibility extremely unlikely.) Kramer demonstrated that the usual daytime compass of birds is the Sun. He trained starlings21 (and subsequently a variety of other birds including pigeons²²) which had a view of the natural Sun to seek food in a specific compass direction, and they readily learned to perform all day long. Apparently the birds could compensate for the Sun's westward movement in the sky. When Kramer deflected the Sun's image with mirrors, the birds were reoriented in the concomitant direction. Finally, when Hoffmann shifted the circadian rhythm of his birds—so that subjective dawn was at noon, say-the birds misread the Sun's azimuth accordingly2 Extensive work with clock-shifted pigeons in the field has confirmed that their preferred daytime compass is solar: a bird shifted by 6 hours will depart from a release site roughly 90° off the homeward bearing on a sunny day²⁴⁻²⁶.

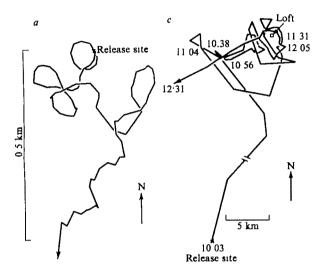
Keeton found that clock-shifted pigeons orient and home normally under overcast skies²⁵; this raises the possibility that rather than using celestial cues perceived through the clouds, they must utilize some back-up compass. Merkel and his colleagues had already shown an exceedingly weak but persistent ability in caged migrants to orient appropriately to the Earth's magnetic field^{27,28}. Keeton discovered that pigeons carrying magnets on their upper backs could orient normally on sunny days, but usually not under overcast skies²⁹. Walcott and Green³⁰, using pigeons equipped with miniature Helmholtz coils on their heads, were able to extend Keeton's results, lending support to the Wiltschkos' suggestion (based on caged migratory robins³¹) that 'north' for pigeons is the direction of the dip lines into the Earth independent of actual magnetic polarity. Given the crucial importance of this 'dip-angle' hypothesis it is remarkable that the Wiltschkos' experiment has not been repeated. Walcott's basic results have been replicated by Visalberghi and Alleva³²

More recently, the Wiltschkos have followed up Keeton's finding that young birds wearing magnets on their first flights cannot orient even with a full view of the Sun²⁹. They have discovered that the Sun compass system must first be calibrated

against the magnetic compass³³. Hence, pigeons kept under permanent clock shift since birth are able to orient and home perfectly well, but are misoriented after being reset to normal time³⁴. The emerging picture of the pigeon compass, then, is that the magnetic sense is innate—based perhaps on the deposits of magnetite recently discovered in pigeons^{35,36} and other navigating species—and is used to calibrate a time-compensated Sun compass³⁴. Having served as an essential reference, the magnetic compass sense seems to be largely or completely ignored whenever solar information is available. A very similar pattern of calibration has now been documented in the ontogeny of orientation in nocturnal migrants³⁷⁻³⁹.

The map sense

In sharp contrast to the steady progress in our understanding of the nuts and bolts of the pigeon compasses, the map sense of



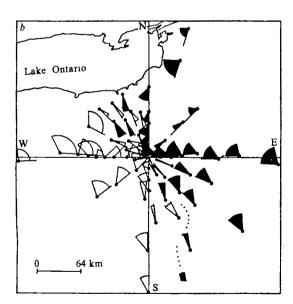


Fig. 1 Behaviour of pigeons while homing. a, After release, pigeons typically circle briefly and then fly off in the general direction of home. b, Release site biases near Ithaca (centre of grid). The mean bearings of pigeons deviate reliably from the homeward direction at almost all locations. There is a strong tendency for the biases of birds released near Ithaca to be clockwise (black) to the east and counter-clockwise (white) to the west of a line running roughly NNW through Ithaca. c, Once near home, pigeons generally use visual landmarks. In this case, however, the bird was wearing frosted contact lenses and so was unable to locate the loft more precisely than a few kilometres. This probably represents the limit of resolution in the pigeon map system. Data from (a) Elsner¹, (b) Windsor², (c) Schmidt-Koenig and Walcott⁷.

these birds has proven exceedingly difficult to elucidate. The first serious theory was put forward by Yeagley^{40,41}. He imagined that pigeons determine their location by means of a bicoordinate grid of magnetic field intensity and Coriolis force. Since the north magnetic pole is about 2,300 km from the north geographic pole (the axis of rotation), the isolines of vertical magnetic field strength and Coriolis forces intersect at about a 30° angle in the northeastern United States. Yeagley's hypothesis has been discounted on both experimental and theoretical grounds⁴².

The second major effort to explain the pigeon's map sense was by Matthews^{43,44}. He proposed that birds could use the Sun's elevation and arc (which they would extrapolate through the sky on the basis of its motion over a brief interval) in combination with their own internal time sense. A pigeon taken south-west, for example, will find the Sun's position unusually high and east (which is to say early) by an amount corresponding to its displacement from the loft. As a compass, the pigeon could use the extrapolated highest point in the arc as a measure of south. A variety of simple observations have made this 'Sun arc' hypothesis seem very unlikely. The two most compelling are the ability of pigeons to home under overcast25 and the birds' consistent interpretation of clock shifts as compass rotations and never as map displacements²⁴⁻²⁶. Hence a pigeon clock-shifted 6 hours early and released at local dawn when its internal clock reads 'noon' ought, according to Matthews, to presume it has been taken a quarter of the way around the world to the west. Such an animal ought to fly east. Instead, it will inevitably depart 90° left of home—west if it has been taken south. Despite its lack of supporting evidence, however, Matthews' hypothesis with its explicit, testable predictions has been the single most important element contributing to our present understanding of both maps and compasses.

At present there are two main map hypotheses, one based on olfaction, the other on magnetism. Both schools of thought offer intriguing and apparently reasonable experimental evidence, and yet neither can, as yet, satisfactorily explain the behaviour of pigeons.

Olfactory maps

The olfaction hypothesis grew out of a curious set of 'palisade' experiments begun by Kramer⁴⁵ and continued by Wallraff⁴⁶ The usual protocol for raising homing pigeons for research is to allow the birds a free view of their surroundings from the loft (presumed to be important for calibration of the celestial compass) combined with daily exercise flights in the immediate vicinity of the loft to build endurance. Finally the birds are taken ever further from the loft for a series of practice releases before testing at longer distances. The practice releases help weed out poor homers. In the palisade experiments, however, the pigeons are never allowed exercise flights or practice releases. As a result, the birds' first release in palisade experiments is usually also their last; such a small proportion is ever found again that data on the directions in which they are found should be discarded. Only the vanishing bearing results can be considered very reliable.

The essential result of the original palisade experiments was that blocking a view of the horizon and the lowest 3° of the sky with wood or even clear glass resulted in birds less well oriented than those permitted an unobstructed view of the Sun, sky and horizon. Blocking the view of the sky with a roof down to just a few degrees above the horizon (and obscuring even that near dawn and dusk to prevent any direct view of the Sun) gave intermediate results (Fig. 2). The conclusion Wallraff drew from these first experiments was that there must be some non-visual "substrate of information spreading in the horizontal plane which is important for homing".

From data derived from later experiments, Wallraff concluded that birds in cages surrounded by opaque plastic louvres which were supposed to block visual cues but permit air to flow through freely were well oriented⁴⁷, but the original data (with the sporadic recoveries omitted) suggest just the opposite

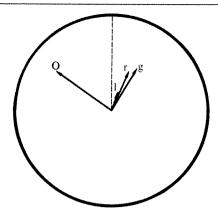


Fig. 2 Results of palisade experiments. By convention, the dashed line is the homeward direction, while the vectors describe the mean angle and degree of scatter among the birds. A vector reaching the circle would represent perfect orientation whereas a vector with no length would correspond to perfectly random data. O, pigeons raised in an open wire aviary (fairly well oriented); r, birds reared in a roofed palisade (well oriented but more scattered); g, pigeons kept in a glass palisade (fairly well oriented but scattered); l, birds raised in a louvred palisade (poorly oriented and highly scattered). Data from Wallraff 46,47.

(Fig. 2). Nevertheless those data, taken together with a recent series of elaborate louvred loft experiments⁴⁸ have been interpreted by Wallraff as implicating an 'atmospheric factor'. In these more recent tests birds in (1) a completely exposed caged loft (the controls) were compared to those raised in lofts surrounded to 10° above the horizon by (2) clear glass, (3) opaque plastic louvres, (4) clear glass on two opposite sides with the other two sides open or (5) wood on two sides and opaque plastic louvres on the other two. Wallraff's conclusion was that birds in lofts with a free flow of air (the controls and loft types 3, 4 and 5) orient better than birds in the loft surrounded by glass. The data in this highly regarded set of experiments are confusing at best, and are made all the more so by the lumping together of bearings at 20 seconds, 40 seconds and at vanishing time after release, and the exclusion of birds judged to be flying too low or not far enough away. Most other researchers consider only vanishing bearings to be reliable. Furthermore Wallraff lumps data from four different release sites with extraordinary release-site biases (based on control releases) ranging from 84° right of home to 149° left. The only procedure by which the confounding effect of these biases can be sorted out is to refer the experimental data to the (very limited set of) control bearings on a site-by-site basis (Table 1). On this basis it seems obvious that both the glass and louvred lofts increase the scatter of departure bearings but have little effect on net homeward orientation. The hybrid lofts, on the other hand, severely disorient their occupants. There is no pattern to the effect even when the direction of the louvred axis is compared to releases along or orthogonal to that axis (see below). In sum, then, Wallraff has shown an extraordinary and mysterious synergism between louvres and wood as well as between glass and open sides. These effects are generally interpreted as disrupting the pigeon map sense. As pointed out below, however, a compass effect is at least as likely to be the explanation, and these releases need to be repeated under overcast skies to see if it is the Sun compass that is being affected.

Despite the many inconsistencies in the various palisade experiments, Papi has followed up the seemingly obvious interpretation that the hypothetical 'horizontal, atmospheric factor' might be odour. Papi and his colleagues believe that birds come to associate the odours borne on the wind with the direction in which the wind is blowing, and so slowly build up an olfactory map of their surroundings⁴⁹. When transported to a release site, then, they have only to sniff the air en route and/or at the site to know the direction of home. Hence the louvred palisades ought not to affect orientation while glass ones might.

How odour could be used by naive pigeons at long distances (hundreds or thousands of kilometres), however, remains a total

mystery^{8,19}. Nevertheless, Papi has conducted a long and ingenious series of highly varied experiments whose results readily lend themselves to this olfaction hypothesis: (1) pigeons whose nostrils have been plugged⁵⁰ or whose olfactory nerves have been cut^{51,52} are poorly oriented at release and home slowly; (2) pigeons taken by different circuitous routes to release sites depart in directions slightly favouring the general direction of the particular detour to which they were subjected^{51,53}; (3) pigeons raised with two different artificial odours added to the wind, one from each of two different directions, and then released with one of the odours applied to their nostrils, are deflected from home as though the direction from which they were released corresponded to the direction from which the odour was carried to them in the loft⁵⁴; (4) the release bearings of pigeons raised in 'deflector' lofts (Fig. 3) whose clear glass or plastic deflectors rotate the incoming wind either clockwise or counter-clockwise are similarly rotated^{55,56}; (5) applying a strong odour to the nostrils to block the perception of local odours disorients the pigeons at release⁵⁷; and (6) the vanishing bearings of pigeons raised in lofts rigged with fans to reverse the natural direction of air flow are either roughly reversed or highly scattered58.

Problems with the olfactory hypothesis

Taken together, Papi's data seem almost overwhelming. Nevertheless, most of the experiments admit of simpler, nonolfactory explanations, or cannot be repeated, or both. One of the problems with the hypothesis comes from the failure of Schmidt-Koenig and Phillips⁵⁹ to detect any ability in pigeons to distinguish natural air (presumably laden with olfactory map information) from pure, filtered air. The technique they used, cardiac conditioning, is extraordinarily sensitive, and has been successfully used in the past to reveal the sensitivity of pigeons to UV light⁶⁰, infrasonic sounds⁶¹, minute differences in air pressure⁶², and polarized light⁶³, though oddly enough it has never succeeded in picking up any sensitivy to magnetic fields (see, for example, ref. 62). Keeton and his colleagues⁶⁴ have repeated the nerve sectioning experiments but found little or no effects on initial orientation. Indeed, when Benevenuti, one of Papi's colleagues, attempted to repeat the experiment, no effect on initial orientation was evident, though homing speed was slower65.

More recently Wallraff⁶⁶ also found that nerve-sectioned birds were less well oriented and slower to home than untreated birds. However, in none of these many experiments by the various groups was homeward orientation abolished. In Wallraff's extensive study, for example, the average mean vector length was an impressive 0.8 for controls, but still a respectable 0.4 for experimentals. Moreover, the orientation of nerve-sectioned birds differed from the controls by only 38° on

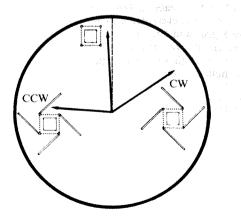


Fig. 3 Design and effect of deflector lofts. The departure bearings of pigeons kept for at least a few weeks in deflector lofts are rotated from those of controls in the same direction as the wind entering the loft is deflected. CW, clockwise loft; CCW, counter-clockwise loft. Data from Baldaccini et al.⁵⁵.

average, while groups of controls at the same sites in two different series of releases differed from each other by 41°. In short, these effects are clearly quantitative rather than qualitative, and probably have nothing to do with destroying the map system per se. Indeed, tracking reveals that the few birds in these experiments usually land and probably spend extended periods grounded⁶⁴, in contrast to the disoriented birds in other experiments who continue gamely flying. It seems likely that the behaviour of both the nostril-plugged and nerve-sectioned birds results from a generalized, distracting trauma. Indeed, when Keeton used nasal tubes to bypass the olfactory chamber and minimize trauma, no disorientation was evident⁶⁷. Moreover, both Schmidt-Koenig and Phillips⁵⁹ as well as Kiepenheuer⁶⁸ have shown that when the olfactory epithelium of a pigeon is sprayed with the potent local anaesthetic xylocaine, initial orientation is normal. Experiments with cardiac conditioning demonstrate that xylocaine-treated birds are unable to respond even to strong artificial odours for about 90 min after treatment⁵⁹.

Papi's detour experiments have also proven difficult to replicate. Keeton^{64,69} found no real effect in almost all cases while Schmidt-Koenig and his colleagues drew a blank in 11 of 12 tries⁷⁰. At the rare sites showing an effect, however, the deflection was relatively consistent. Of course the olfaction hypothesis is by no means the only explanation for a detour effect. Similarly, neither Keeton's group ^{64,71} nor Schmidt-Koenig's⁷⁰ could find that applying masking odours to the beaks and nostrils of pigeons had any predictable effect.

Of all the non-surgical manipulations reported by Papi's group, only the deflector loft has worked consistently elsewhere. Both Kiepenheuer⁷² and Keeton's group^{73,74} have obtained consistent rotations of departure bearings, but there seems no reason to suppose that these dramatic effects have anything to do with odour. Wallraff, for example, has pointed out that the results from his many louvred palisade experiments contradict the olfaction hypothesis. In the heroic series of experiments summarized in Table 1, releases from along the axis of the louvres or open ends of loft types 4 and 5 ought to have yielded far better orientation than releases from along the orthogonal axis. In fact, there was no difference: the mean vector length and angular error for on-axis releases was 0.47 and 99° versus 0.45 and 99° off-axis⁴⁸. Similarly, Kiepenheuer found the deflector lofts worked just as well on birds with plugged nostrils or with xylocaine treatment⁶⁸, implying that the deflection is not a result of rotated windborne odours.

The greatest difficulties with both the palisade and deflector loft experiments arise from the uncontrolled effects that the various clear and opaque plastic and glass elements certainly have on the transmitted and/or reflected UV and polarized light which pigeons perceive. The UV-polarized light of the blue sky is well known to be of supreme importance in the orientation of a variety of animals, most notably the honey bee, and all of the various plastic and glass elements are opaque to UV and greatly alter the degree and direction of polarization. Even more problematical are the reflections each would inevitably generate. Following up on these worries and the suspicious observation that the deflector effect is very slight or even absent under

Mean error Sample Mean vector Type of loft length (re: controls) size Control: Wire loft 0.39 210 Glass loft 340 774 0.14 569 28° Louvred loft 0.24(4) Glass and open loft 289 0.24141°

177°

521

0.22

Table 1 Comparison of data on differing release sites

Data calculated from ref. 48.

Wood and

louvred loft

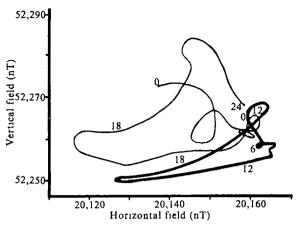


Fig. 4 Daily patterns of magnetic field intensity. On normal days (heavy line) the pattern is relatively regular. (The numbers by the curves are hours GMT.) On 'stormy' days, however, the pattern is irregular (thin line). These curves are based on hourly means recorded by the US Coast and Geodedic Survey and Fredricksburg, Virginia in September 1973. The hourly means suppress the frequent but brief 100 nT pulses which accompanied this storm.

overcast skies, two of Keeton's colleagues, Waldvogel and Phillips, designed a clever deflector loft which rotates wind one direction while it reflects incoming visual cues the other way. The release bearings of the pigeons raised in these lofts are strongly rotated in accordance with the visual cues, demonstrating that the rotation of visual cues is more important in creating the phenomenon⁷⁵. Hence, the deflector loft effect is probably a recalibration of the pigeon compass rather than of the map sense.

A more detailed look at these new data⁷⁵ makes it clear that, as Kramer and Wallraff had already shown, no direct view of the Sun (or its reflection) is necessary for recalibration. This observation points to the probable use of polarized light, perhaps along the lines described by Brines⁷⁶. The most likely calibration component would be the prominent band of horizontal polarization which defines the solar and antisolar meridian, and which Brines and Gould have found to play an important role in bee navigation^{77–79}. Similarly, Able has found that when nocturnal migrants calibrate their compasses at dusk for their upcoming flight, altering the skylight polarization above them rotates the birds' compasses⁸⁰. It seems likely, then, that polarization patterns, so long known to be crucial to the orientation of invertebrates, amphibians, and fish, play a hitherto unappreciated role in birds.

The olfactory hypothesis, though initially very exciting, has lost some of its lustre as a result of these many problems. It may yet turn out to be true, and two experiments—training pigeons to strong, artificial odours in the loft⁵⁴, and reversing the natural wind direction in the loft⁵⁸—still stand. Even here, however, doubts intrude: (1) no one has yet repeated either; (2) Papi found that homing speeds in the strong-odour experiment were unaffected⁵⁴; (3) even Papi's group finds that rearing birds with no olfactory experience whatsoever does not affect initial orientation⁸¹; and finally (4) the maze of 'clear' plastic and glass used to construct these elaborate lofts must introduce just the sort of visual problems which may well account for the deflector effect. Of course there remains the possibility that Italian homing pigeons have a map system altogether different from that of German and American birds; and strain-specific differences in homing performance and initial orientation are well known (W. T. Keeton, personal communication). However, neither Kiepenhauer⁷² nor Wallraff^{11,12} have been able to find any major or consistent differences in orientation between German and Italian pigeons.

Magnetic maps

The major alternative to the olfaction hypothesis at the moment, put forward independently by Walcott⁸², Gould⁹, and Moore⁸³,

is based on the gradual and basically regular change in the dip angle and strength of the Earth's magnetic field. The field strength of the Earth at 40°N latitude in the US is about 0.5 G (50,000 nT). The field has several sources: by far the strongest is generated by the Earth's core, while a much weaker effect arises inductively from the movement of ions around the Earth by the jet streams. Because the daily heating and cooling of the atmosphere displaces the jet streams north and south, a moreor-less regular circadian variation of 30-50 nT is observed on the ground (Fig. 4). The total magnetic field of the Earth in the northeastern United States increases at about 3-5 nT km⁻¹ to the NNW, the vertical strength rises roughly twice as fast, and the dip angle increases at about 0.01° per km.

To account for the accuracy displayed by pigeons wearing frosted contact lenses⁷, a seemingly impossible sensitivity of 10-30 nT or 0.03° would be necessary; but just such sensitivities can be inferred from the behaviour of birds. After solar flares, for example, enormous numbers of extra ions appear in the jet streams, causing irregular changes in the strength of the Earth's field ranging from 10 to 1,000 nT (Fig. 4). These 'storms' have roughly dosage-dependent effects on birds⁸⁴. For example, the release-site bias of Ithaca pigeons released at Campbell, New York, is rotated up to 40° counter-clockwise (in addition to the usual 12° bias) during moderate storms (Fig. 5a). (Regrettably, no data on how scattered the birds' vanishing bearings were during such storms has been published.) A retrospective analysis of 18 years of homing pigeons races in Italy 85,86 confirms a strong negative correlation between homing speed and the sunspot activity which is responsible for such storms, and Yeagley pointed out a similar relationship in American races⁴². Similarly, Moore observed that the increasing levels of magnetic storm activity increased the angular deviation of free-flying nocturnal migrants⁸⁷, while Southern found a substantial effect on the orientation of gull chicks88. These various observations imply a sensitivity of 10-30 nT. Since even a 1,000 nT storm could not, in the most extreme conditions, rotate a compass needle even 2°. and since the effects on pigeons and gulls are observed when the sun is clearly visible, the phenomenon is more likely to be related to the map sense.

Although the effects of solar activity on pigeon orientation are well documented and intriguing, naturally occurring variations in magnetic topography provide a static and therefore more convenient index to magnetic sensitivity. To use a magnetic map, pigeons would have to extrapolate the local rate and direction of magnetic gradients (inferred either during exercise flights or during transport to the release site) in order to judge the distance and direction of displacement. Hence any slight nonlinearity in values at the loft ought to result in a vague but

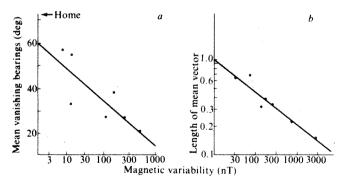


Fig. 5 Effect of small magnetic field disturbances on homing pigeons. a, Birds released at Campbell, New York normally show a site bias of 12° CCW. This bias is exaggerated during magnetic storms. Storm data represents a 12-hour average of the most active magnetic component as recorded at Fredricksburg. Data from Keeton et al. 84. b, Vanishing bearings of birds released at magnetic anomalies are more scattered in proportion to the strength of the anomaly. The data, from Walcott 89, are based on the magnetic variability in the homeward direction over the first kilometre.

systematic pattern of errors at release sites (imposed, of course, on any site-specific deviations) roughly aligned with the general direction of the regional magnetic gradients. This seems to be the case. Figure 1b, for example, reveals a pronounced tendency for site biases around Ithaca to be clockwise east of the NNW line of magnetic field gradients, and counter-clockwise to the west. It is as though 'pigeon' Ithaca is NNW of geographic Ithaca. Similarly, the pattern of biases around Schmidt-Koenig's former loft at Frankfurt (where the gradient runs west) and several locations mapped by Wallraff consistently fall into this pattern. As would be expected in some cases (one of Wallraff's as well as Walcott's loft in Lincoln, Massachusetts), the clockwise/counter-clockwise pattern is reversed as though the loft is magnetically south of its true location along the axis of the regional field gradients.

The Earth's magnetic field also has permanent localized anomalies, generally created by areas of high magnetic permeability such as iron deposits. These provide a favourable path for magnetic lines of force, concentrating the lines and thereby raising the local field strength. Walcott has carried out an extensive series of releases at anomalies of various strengths and finds a clear relationship between the magnitude of the anomaly and the degree of scatter in vanishing bearings (Fig. $(5b)^{89}$. Tracking of birds from normal (Fig. 6a) and anomalous sites (Fig. 6b) on sunny days illustrates the dramatic and longlasting nature of the effect. It seems clear that these small irregularities in magnetic field strength leave birds with almost no idea of where they are; and the persistence of the effect for a time after they have escaped from the anomaly suggests a time-averaging processing strategy similar to that of bees⁹¹. It is also worth noting that the 10-30 nT level at which effects begin to be evident corresponds to the predicted threshold for a magnetic map sense based on the accuracy displayed by the birds wearing frosted lenses⁷.

Along the same lines Wagner ^{92,93} has found a strong tendency for pigeons to respond to magnetic slopes of about 30 nT km⁻¹. His birds flew down slopes regardless of home direction—a sensible strategy since gradients of more than 5 nT km⁻¹ indicate an anomaly—and, since virtually all anomalies are the result of elevated local field strength, down is almost always the way out to the magnetic 'plains' where more reliable map readings would be possible (see Fig. 6). (Similar though less well documented effects of magnetic topography have been reported by both Talkington ⁹⁴ and Graue ⁹⁵.) Moreover, such a 'fly-down' strategy could explain the mysterious difficulty of nearly all Ithaca pigeons to orient accurately at the Jersey Hill fire tower in western New York state. The few birds which have been tracked from aircraft wander about within a few kilometres tracing out the limits of what is, to a first approximation, a magnetic 'valley'.

Further suggestive evidence in favour of a magnetic map sense comes from three diverse sets of experiments by Kiepenheuer⁹⁶, Wiltschko's group⁹⁷, and even Papi and his colleagues⁹⁸. In each case an alteration of the magnetic field felt by the birds on the way to the release site resulted in a significant change in orientation. These recent results are in marked contrast to older experiments (cited earlier) with less completely controlled fields which found no effect. The exact reason for these differences is not clear.

Problems with the magnetic hypothesis

Encouraging as all these data seem, the magnetic map hypothesis faces severe problems. The first is that two axes are needed for bicoordinate navigation. The magnetic parameters available are total field strength, vertical strength, horizontal strength, declination (the deviation of the horizontal vector from true north), and inclination (the dip angle). Declination is useless since it requires knowing true north at the release site even under overcast skies; and both inclination and total field strength use up the same two components: vertical and horizontal strength. Hence, birds would have either to compare horizontal against vertical or total field strength which would form a grid in the northeastern United States intersecting at

about 20°. If pigeons were using such a grid, then departures ought to be more accurate and less scattered to the ENE and WSW. In fact, only a slight trend of this sort is apparent in the data. Of the two other possibilities, total intensity and dip angle (the ratio of vertical and horizontal intensities), only the latter seems likely: there are areas with large, gentle variations in field intensities—a result probably of either weak, large-scale differences in ground permeability to magnetic fields, or strong but deep anomalies—which would overwhelm any simplistic

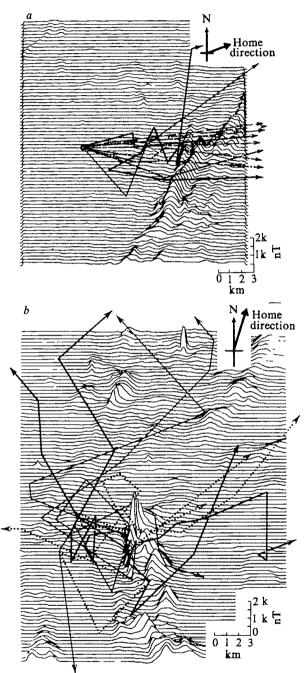


Fig. 6 Flight paths of pigeons away from release sites. Total magnetic intensity (the only parameter available from the aeromagnetic surveys by the US Geological Survey upon which these illustrations are based) increases steadily at 4 nT km⁻¹ to the NNW, creating an inclined plane. Only the anomalies in this pattern have been drawn here and are seen as 'hills'. a, Pigeons released from a magnetically normal site near Worchester, Massachusetts depart directly away, displaying the slight clockwise bias typical of this site. b, Birds released at the magnetic anomaly at Iron Mine Hill, Rhode Island, on the other hand, are thoroughly disoriented. At this site the field strength is approximately 6% high. Data from (a) Walcott (personal communication) and (b) Walcott⁸⁹; drawings from Gould⁹⁰.

attempt to make sense of regional patterns of total field intensity but which might not affect the horizontal/vertical ratio. This ratio, the inclination or dip, is precisely the measure employed for magnetic compass orientation in robins³¹ and probably pigeons³⁰. But if birds must abandon total intensity for dip, they must be able to measure true vertical—presumably gravity—as a reference angle with great precision (that is, to within 0.03°, a difficult but not impossible task). Perhaps this could help explain some of the puzzling, weak gravitational effects reported by the Keeton group⁹⁹. To further confound matters, most magnetic anomalies, created as they are by iron deposits, are gravity anomalies as well.

Another apparent problem is that strong magnets and Helmholtz coils normally do not destroy orientation on sunny days^{29,30,100}. How could pigeons possibly measure such minute field changes in the face of strong static fields? Interestingly, however, the vanishing bearings of pigeons given such strongfield treatments correspond to those seen on days of severe magnetic storms¹⁰¹—suggesting a map effect. Moreover, when Walcott released magnet-equipped pigeons at anomalies, the effect of those small geographical disturbances persisted 102 indicating that these small perturbations were being perceived through the strongly altered background. Taken together, both the data and theoretical considerations suggest that any magnetic map detector must attend solely to the small changes between and around both loft and release site, and ignore the far larger but utterly useless background field which is essential for the cloudy day compass system. A further constraint is that such a map system must have a fairly long time constant since (1) pigeons can usually fly through anomalies with little effect, (2) the birds are not disoriented by wing-mounted magnets which would create an alternating background field 38,39,103 (3) they seem less disturbed by the brief but powerful 'pulses' in magnetic field storms than by longer-term changes in background field, and (4) homing pigeons do not recover from releases at anomalies until they have been out of the field for some time (Fig. 6b).

A final problem for any magnetic map system is how the nervous system could possibly measure such small intensity variations. Although Kirschvink and Gould have shown that the vast numbers of single domains of magnetite discovered in pigeons could produce a resolution better than 1 nT¹⁰⁴, whether either these calculations or the magnetite have anything to do with pigeon navigation remains to be seen.

The real difficulties with the magnetic map hypothesis, then, are threefold. (1) It has no satisfactory explanation of the necessary second coordinate (it could be magnetism again, odour, infrasound, the Sun (though obviously not on cloudy days), or even Coriolis force). (2) It is based almost entirely on correlations rather than direct, controlled experimental manipulations (the effects of strong, artificial field alterations during transport, for example, ought to have generally unpredictable or even negligible effects). And (3) there is as yet no convincing explanation of either the sensory basis of magnetic field perception or the processing of that data to extract map information. Given the evidence for both olfactory and magnetic effects, it would be attractive to suppose that both answers are correct: perhaps, by analogy with the solar and magnetic compass of pigeons, birds can use either as the situation demands; or, both might be used simultaneously. If the first possibility were correct, we would expect that repeated releases at magnetic anomalies ought to force pigeons into using the alternative olfactory map system. However, since Walcott has released the same pigeons repeatedly at different anomalies, and such birds simply do not improve, the inference is that birds must lack an alternative system. Of the possibility that olfactory and magnetic information must be used together, one can only say that it seems at least as plausible as that they use magnetic information alone or with some unknown factor.

It is ironic that, more than three decades after Yeagley's original hypothesis, we are still largely in the dark and, for all we know, his explanation may yet turn out to have been rather close

to the mark. We probably now have nearly all the pieces of the puzzle before us (and, doubtless, several utterly irrelevant ones as well) but for the immediate future, at least, the nature of the animal map sense seems likely to retain its status as the most

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I thank C. Walcott, C. G. Gould and D. R. Griffin for their contributions, and the NSF for its support through grant BNS 78-24754.

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RTICLES

Pancake detonation of stars by black holes in galactic nuclei

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Recent efforts to understand exotic phenomena in galactic nuclei commonly postulate the presence of a massive black hole accreting gas produced by tidal or collisional disruption of stars. For black holes in the mass range 10^4 – $10^7 M_{\odot}$, individual stars penetrating well inside the Roche radius will undergo compression to a short-lived pancake configuration very similar to that produced by a high velocity symmetric collision of the kind likely to occur in the neighbourhood of black holes in the higher mass range $\geq 10^9 \, M_{\odot}$. Thermonuclear energy release ensuing in the more extreme events may be sufficient to modify substantially the working of the entire accretion process.

MANY of the most plausible models for explaining spectacular energy release phenomena in galactic nuclei are based on the release of gravitational energy by gas accreting on to a very massive central black hole¹. A likely mechanism² for providing the gas necessary to fuel such a model would be the breakup of ordinary stars, either by the Roche tidal effect in the case of a black hole with mass M below the Hills³ limit, $M \leq 10^8 \, M_{\odot}$, or else by the effect of collisions in the case of a more massive black hole with a sufficiently dense surrounding star cluster. Gas release by tidal or collisional breakup (as well as by mechanisms such as supernovae and ordinary stellar winds) may also be important at other relatively quiescent stages in the history of a galactic nucleus containing a black hole, that is at times when the most observationally spectacular phenomena (such as quasars) are absent.

This article draws attention to a neglected effect which could be significant in many situations where tidal or collisional disruption occurs, namely that in the more extreme events (which may constitute a not negligible fraction of the total) the disruption will be preceded by a short-lived phase of high compression to a roughly pancake shaped configuration in which the density and temperature may rise enough to detonate effectively some significant fraction of the available thermonuclear fuel.

A very flat pancake type configuration will be formed briefly by any nearly head-on collision between roughly similar stars at very high relative velocities (analogous pancake formation being experimentally familiar in the microscopic context of very high energy proton-proton collision). We have in mind in particular the kind of event that is likely to occur near a black hole with the very large mass $M \ge 10^9 M_{\odot}$ that is thought⁴ to be necessary to account for the most extreme quasar phenomena. Beyond 0.1 pc from such an object, typical stellar collisions will have velocities ≥10⁴ km s⁻¹, thus exceeding by a factor $\beta > 10$ the minimum of the order of 10^3 km s^{-1} needed for disruption. Detailed studies of high velocity (≥10⁴ km s⁻¹ collisions will require an elaborate hydrodynamic treatment, with allowance for shocks, of the kind already available⁵ for the intermediate velocity (≈10³ km s⁻¹) collisions in which a moderate degree of flattening is already present. However, a preliminary idea of the effects to be expected may be obtained from consideration of the simpler but otherwise very similar configuration that arises during tidal disruption of a single star, whose evolution can be followed approximately6,7 in terms only of ordinary differential equations using an adiabatic affine star model which, although highly idealized, can, nevertheless, be expected to provide a qualitatively valid description of the behaviour of the stellar core before, if not after the instant of maximum compression.

The phenomena of tidal disruption of a self gravitating body passing within the Roche radius

$$R_{\rm R} \approx M^{1/3} \rho_{\star}^{-1/3} \tag{1}$$

(where ρ_* is its characteristic central density) has been studied for over a century, but mainly in terms of an incompressible fluid model. It does not seem to have been realized that adequate allowance for compressibility leads to the prediction that a star penetrating deeply within the Roche radius will pass through a phase of compression to a highly flattened pancake configuration, closely similar, in both spatial and temporal characteristics, to that produced by a symmetric collision involving a pair of stars. It turns out that the effect of a collision with stellar velocities exceeding the central characteristic velocity (which is a few hundreds of km s⁻¹ for a typical main sequence star) by a factor of the order of β can be roughly simulated by a single star following an orbit that penetrates to within a pericentre radius R_p given in order of magnitude by

$$\beta \approx R_{\rm R}/R_{\rm p} \tag{2}$$

(a value β of the order of 10 thus being sufficient to reproduce the effect of a collision with relative velocity of the order of $10^4 \,\mathrm{km \, s^{-1}}$). Not only can the study of thermonuclear reactions in such a tidally produced pancake give a first rough indication

of what may occur in a high velocity stellar collision, but it would also appear to be an astrophysically interesting phenomenon in its own right in the context of a black hole in the moderate mass range $10^4 M_{\odot}$ to $10^7 M_{\odot}$ for which it is possible to obtain a large value of β without the star either being penetrated by or swallowed by the black hole. The potential importance of this phenomenon stems from the fact that deep penetration is much less improbable than would be expected from purely geometric considerations. Just as the probability of high velocity collisions deep in the potential well of an $M \ge 10^9 M_{\odot}$ black hole is enhanced far above what would be proportional to the corresponding volume (by an amount whose exact calculation is difficult) in consequence of the well known cusp effect in the stellar density distribution (see ref. 8), so, similarly, the probability of penetration of an individual star deep within the tidal field of a more moderate sized black hole will also be much higher than would be deduced from the corresponding purely geometrical cross-section, by an amount that can be relatively easily estimated from the characteristics of the individual approximately parabolic orbit. We conclude that the fraction of all tidally disrupted stars with penetration factor exceeding a given value β will be of the order of magnitude of β^{-1} .

Extent and duration of compression

To derive equation (2) it is sufficient to see that the tidal force on the star (which is inversely proportional to the cube of the radial distance R from the hole) will rapidly dominate the internal pressure and self-gravitational forces on the star as it begins to penetrate substantially within the Roche radius R_R (the latter being characterized by the condition that the tidal and internal forces have comparable magnitude). In these circumstances it will become a good approximation to treat the individual particles of the star as undergoing free fall in the external gravitational field on orbits of approximately parabolic form in planes all passing very nearly through the perihelion point on the centre of mass trajectory. These planes will be confined roughly within an angle

$$\alpha \approx R_{\star} (R_{\rm p} R_{\rm R})^{-1/2} \tag{3}$$

where R_* is the characteristic radius of the star in its approximately unperturbed state before passage across the Roche radius, as given in terms of the stellar mass M_* by

$$R_{*} \approx M_{*}^{1/3} \rho_{*}^{-1/3} \tag{4}$$

Thus in the centre of mass frame of the star the particles will effectively be accelerated towards the orbital plane, with maximum velocity, u say, of the order of αv where v is the orbital velocity at the pericentre, that is

$$u \approx \alpha \left(\frac{GM}{R_{\rm p}}\right)^{1/2} \tag{5}$$

Hence we immediately see that the relative velocity u of what may be described as the collision of the star with itself will have the required form

 $u \approx \beta \left(\frac{GM_{\star}}{R_{\star}}\right)^{1/2} \tag{6}$

where the factor $(GM_*/R_*)^{1/2}$ is interpretable as the characteristic velocity of sound in the core of the star.

When matter becomes sufficiently highly compressed, the free-fall treatment will, of course, cease to be valid: as the mass centre passes through the pericentre, the tidal forces, although high, will be overtaken by a very sharp last minute rise in the pressure forces, which will cause the star to bounce back towards a more isotropic configuration. Note that stellar deformation in directions in the plane of the orbit will not have had time to become important at the instant of passage through the pericentre (the 'tube of toothpaste' extrusion effect that is familiar from the incompressible case cannot have developed significantly at this stage) so the maximum degree of compression of the resulting pancake can be estimated directly from the

requirement that the kinetic energy of the 'self-collision' be entirely converted into internal energy of the gas at the instant of turn around. If this energy is predominantly thermal, as will be the case in main sequence stars, the maximum central temperature Θ_m in the pancake will be given in terms of its value Θ_{\star} in the unperturbed state of the star by

$$\Theta_{m} \approx \beta^{2} \Theta_{\star} \tag{7}$$

This formula will also be valid when the predominant internal energy is that of a non-relativistic degenerate electron gas, which scales proportionally to the non-degenerate thermal energy of the gas of positive ions. As long as the gas remains non-relativistic the corresponding maximum central density $\rho_{\rm m}$ will be given by

$$\rho_{\rm m} \approx \beta^3 \rho_{\star} \tag{8}$$

and the duration τ_m of the phase of maximum compression will be given by

$$\tau_{\rm m} \approx \beta^{-4} (G\rho_{\star})^{-1/2} \tag{9}$$

where $(G\rho_*)^{-1/2}$ is the free-fall time scale of the star (which is automatically the same as the orbital time scale at the Roche radius R_R) whose value is of the order of 10^3 s for small main sequence stars.

Although the above arguments are crude we have confirmed them (see Fig. 1) by explicit numerical integration of the equations of motion governing an affine star model (in which the constant density layers are treated as retaining an ellipsoidal form) which represents the simplest and most natural extension of the incompressible fluid model (on which most previous numerical studies of tidal forces have been based). While probably at least qualitatively adequate during the compressive phase, such a treatment can be expected to be invalidated during the phase of subsequent expansion by the effect of shocks, whose potential importance in the envelope (as opposed to the central regions with which we are concerned here) has been pointed out by Lidskii and Ozernoy⁹, who seem to have been the first to appreciate the significance of the tidal field's initial tendency to cause compression.

Thermonuclear detonation

It is evident from equation (7) that a quite modest Roche penetration factor $\beta \approx 10$ can raise the temperature of a main sequence star from its usual value $\Theta_{\rm m} \approx 2 \times 10^{9} \, {\rm K}$ to near the value $\Theta_{\rm m} \approx 2 \times 10^{9} \, {\rm K}$ at which (according to the standard formula given for example by Fowler *et al.*¹⁰) helium combustion by the triple- α reaction proceeds at a maximum rate for a given density, the relevant time scale being given (in c.g.s.) by

$$\tau_{\alpha} \approx 10^{11} (\rho_{\rm m} X_{\alpha})^{-2} \tag{10}$$

where the helium mass fraction X_{α} will have at least the cosmological value $X_{\alpha} \approx 1/4$. As the energy that can be obtained from helium combustion is only of the same order as the thermal energy (borrowed temporarily from the gravitational field) already present, the condition $\tau_{\alpha} \leqslant \tau_{\rm m}$ is all that is required for a substantial fraction of this energy to be released. As the values $X_{\alpha} \approx 1/4$ and $\beta \approx 10$ lead roughly to $\tau_{\rm m}/\tau_{\alpha} \approx 10^{-5} \rho_{\star}^{3/2}$, we see that the efficiency of helium detonation depends primarily on the central density of the star, being reasonably high only when ρ_{\star} approaches the order of 10^3 g cm⁻³. By coincidence this is roughly the upper limit for main sequence central densities, being obtained near their lower mass limit, $M_{\star} \approx 10^{-1} M_{\odot}$.

The foregoing leads us to conclude that the most favourable conditions for efficient helium detonation will occur in conceivably very numerous (see ref. 11) 'dark dwarf' stars that are just too small to reach the main sequence but which, nevertheless, maintain central temperatures $> 10^6$ at densities ranging up to 10^3 g cm⁻³ during time scales of the order of 10^9 yr (see ref. 12). To reach $\Theta_m \approx 10^9$ K such a star needs perhaps twice as large a penetration factor β as in the main sequence case (with a correspondingly halved probability), but as a result

 $\rho_{\rm m}$ will be raised from a main sequence maximum of the order of $10^6~{\rm g~cm^{-3}}$ to a value that can exceed $10^7~{\rm g~cm^{-3}}$. At such densities the degenerate electrons will enter the relativistic regime, so that equations (7)–(9) will need modification, but allowance for this seems to confirm the conclusion that $\tau_{\rm m}/\tau_{\alpha}$ attains a value of the order of 1.

The carbon-12 formed in such a detonation process will immediately undergo further processing by α -capture and proton-capture reactions (whose details depend very sensitively on M_* and β) so that the total energy release may exceed the internal binding energy of the star by a factor of the order of 10². Thus instead of forming a diffuse cloud weakly bound to the black hole as in the non-violent disruption scenario described by Hills³, the gas will be ultimately liberated from the star in the form of a cloud expanding at a rate far in excess of the velocity of escape from the black hole. Any discussion of detailed consequences is beyond the scope of this article. We mention only that the immediate effect of the pancake detonation will be to accelerate the matter orthogonally to the orbital plane, but that subsequent radioactive energy release may lead to slingshot ejection of a fraction of the matter at very high velocities in the direction opposite to that along which the star originally approaches the hole, and to the corresponding capture by the black hole of comparable fraction of the matter in very tightly bound cloud or disk, with thermal or orbital speeds $> 10^4$ km s⁻¹. It is even conceivable that a self-sustaining hot C-N-O or rapid proton capture 13 hydrogen burning process may be set off in the expanding cloud, with even more spectacular consequences, but only a careful hydrodynamic treatment with adequate treatment of shocks will be able to check this. Analysis of the overall scenario resulting from interaction of gas from many disrupted stars will be even more complicated but we can conclude that the likely detonation of a fraction of the order of 1% (arising from say a 10% fraction with sufficiently high penetration factor β , of which a less easily estimable fraction, perhaps also of the order of 10%, may lie in the susceptible mass range) will probably be sufficient to modify significantly the overall energetics of the entire gas release process. (Analogous conclusions are probably valid for the more complicated situation resulting from collisional, as opposed to tidal, disruptions in the neighbourhood of much more massive black holes: the unfavourable statistical factor resulting from the probable necessity that both participants in the collision should lie in the susceptible mass range seems likely to be compensated by the enhanced probability of very high velocities due to the cusp effect.)

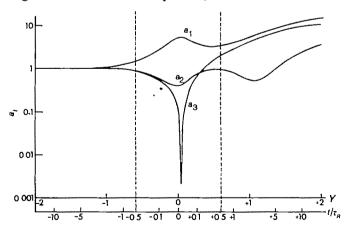


Fig. 1 The numerically calculated magnitudes of the principal axes a_1 , a_2 , a_3 (a_3 being orthogonal to the orbital plane) of a polytropic ($\gamma = 5/3$) affine (that is, ellipsoidally deformed) star model are plotted on a logarithmic scale as a function of the distance Y from the axis of a parabolic trajectory with Roche penetration factor $\beta = 10$. The corresponding time t, normalized as a fraction of the stellar oscillation time scale $\tau_R = (G\rho_*)^{-1/2}$, is indicated on a separate scale below, the approximate moments at which the star enters and leaves the Roche radius R_R being indicated by dotted lines.

Although the triple- α reaction we have been considering provides the only opening for significant thermonuclear release from the compression of population II stars (consisting almost exclusively of hydrogen and helium) it has been drawn to our attention that if the stellar population in the galactic nucleus has composition similar to that of population I stars, such as the Sun, then energy release on an individually more modest scale, but in a much larger fraction of the disrupted stars, can result from C-N-O cycle reactions. It is not possible to carry through a complete C-N-O cycle during the short lifetime of the pancake configuration because some of the steps inevitably involve slow weak decays. Nevertheless, the first highly temperature sensitive proton-capture stages (together with the later disintegration of the products) can liberate several times the binding energy of a star of solar type, if the temperature in the pancake rises above a few times 108 K. As this effect is much less density sensitive and requires a lower Roche penetration

Received 17 September 1981, accepted 27 January 1982.

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factor ($\beta \approx 5$ would suffice) than the triple- α reaction, it will occur in a much larger fraction of the stars involved. Hence, although the individual events are much less spectacular, the total energy release from this process can (when averaged over all disrupted stars) provide a contribution to the overall energetics that is no less important than that arising from helium detonation. Indeed for a black hole near the Hills mass limit (where the larger penetration factors required for triple- α detonation are not possible) energy release from proton-capture will be the dominant contribution. The net effect will presumably be to convert the gas from the disrupted stars into an outgoing wind, leaving only a small fraction to be accreted by the hole.

We thank many colleagues, particularly S. Bonazzola, S. Collin, T. Damour, D. Péquignot, M. Rees, J. Schneider, H. Sol and C. Zaidins, for helpful discussions.

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The rare earth elements in seawater

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The depth distributions of La, Ce, Nd, Sm, Eu, Gd, Dy, Er and Yb in the oceanic water column are used to evaluate the marine geochemical cycle of the rare earth elements and their application as water-mass tracers.

THE chemistry of the rare earth elements (REE) makes them particularly useful in studies of marine geochemistry. They are an extremely coherent group so that their relative abundances can be used to deduce their sources in sedimentary deposits¹. However, certain subtleties of this chemical similarity give additional important information. First, there is an ordered variation in the stability constants of REE complexes which means that the partition of rare earths between marine phases may fractionate the light relative to the heavy rare earths. Second, although the REE are predominantly trivalent there are exceptions: cerium can exist as Ce (IV) and europium as Eu (II), and these elements may fractionate from the 3+ REE as a function of redox potential. Third, some of the REE are weakly radioactive. In particular the decay of 147 Sm $(t_{1/2} =$ 1.06×10¹¹ yr) to ¹⁴³Nd coupled with the fractionation of Sm/Nd in the continental crust relative to the mantle means that the ¹⁴³Nd/¹⁴⁴Nd ratio may be used to constrain the sources

For these reasons the REE (and, recently, Nd isotopes) have been used to assess the origins and depositional environments

of modern sediments and have been used as a suite of chemical indicators of oceanic change²⁻¹². The full exploitation of such applications requires a detailed understanding of the geochemical interactions of the REE in oceans and marine deposits. Our understanding of geochemical cycles in the oceans has increased dramatically as a result of recent measurements of the distributions of certain trace elements in the oceanic water column¹³⁻²². In such cases, problems of sampling, contamination and analysis have been overcome to produce "oceanographically consistent" profiles which can be interpreted both in terms of known hydrographical and chemical features and also in terms of processes of element supply and removal such as organic matter regeneration, aeolian deposition, hydrothermal input, diffusion from bottom sediments and particle scavenging. No systematic study has yet been made of the distributions of the rare earths in the oceanic water column, the only measurements of the concentrations of the REE group in seawater being on isolated samples by Balashov²³, Goldberg²⁴ and Hogdahl²⁵ during the 1960s and by ourselves recently⁶, together with recent determinations^{7,8} of Nd and Sm. For this

Depth (m)	La	Ce	Nd	Sm	Eu	Gd	Dу	Er	Yb
0	36.7	66.3	34.3	6.01	0.615	5 .5 9	5.00	3.63	3.15
100	13.0	16.8	12.8	2.67	0.644	3.41	4.78	4.07	3.55
200	17.0	22.3	15.8	4.52	0.849		5.31	4.62	4.07
600	22.5	18.4	19.7	3.86	0.796	4.85	5.41	4.58	4.14
700	25.2	24.7	21.9	4.23	0.757	5.23	5.43	4.57	4.07
900	20.8	9.64	21.1	4.32	0.823	5.20	5.61	4.94	4.66
1,000	_	20.8	22.8	4.51	1.01	_	6.00	_	
1,500	22.8	9.71	19.0	3.72	0.954	5.31	6.03	5.30	4.99
2,500	29.4	26.1	25.0	4.75	0.895	7.19	6.10	5.09	4.79
3,000	32.6	19.3	25.4	4.69	0.987	5.80	6.14	5.33	5.21
4,500	54.4	55.1	45.8	8.25	1.22	8.27	6.83	5.34	5.16

reason we have determined the concentrations of nine rare earth elements in a vertical profile from the North Atlantic Ocean.

The seawater samples were collected in February 1979 during RRS Shackleton cruise 1B/79 from 28°01'N 25°59'W in the Cape-Maderia Abyssal Plain, water depth 5,250 m (also the site of GEOSECS station 115). The hydrography of the station (see also Fig. 4a) consists of a mixed layer of ~100 m, North Atlantic central water down to ~900 m, a diffuse lens of Mediterranean water with its core at ~1,200 m but influencing the potential temperature-salinity relationship to 2 km or more, below which is North Atlantic deep and bottom water. Our mass-spectrometric isotope dilution method for seawater analysis has been described previously, although some modifications have been made. Our latest analytical scheme is, briefly: the 0.4-µm filtered samples of ~501 are acidified to pH 2 and the REE spikes added. After equilibration, the REE are concentrated by co-precipitation with ferric hydroxide, separated from the sea salts by cation exchange and from Ba by mixed solvent anion exchange. They are analysed as a group in the sequence

Eu-Yb-Ce-Sm-Nd-La-Er-Dy-Gd by selective ionization (VG Isomass 54E mass spectrometer) with on-line computing²⁶. Analytical precision and accuracy determined by running standard solutions is typically $\pm 1\%$ (2σ) or better with ratios of sample REE to reagent plus column blanks of $>10^3$.

REE distribution in the oceanic water column

The REE are present at extremely low concentrations in seawater (Table 1), $\sim 10-70\times 10^{-12}$ mol kg⁻¹ for La-Nd, $\sim 0.5-1\times 10^{-12}$ mol kg⁻¹ for Eu and $\sim 3-8\times 10^{-12}$ mol kg⁻¹ for Sm-Yb. Despite these ultra-trace concentrations, factors such as contamination do not seem to have been an insurmountable problem (although some unascribable scatter is present) and the results display non-random vertical distributions (Fig. 1).

The profiles of all the REE are characterized by: (1) a deep-water enrichment, all showing significant increases in concentration towards the bottom; (2) extrema in the mid-water column ~1,000 m; (3) low but non-zero concentrations at the

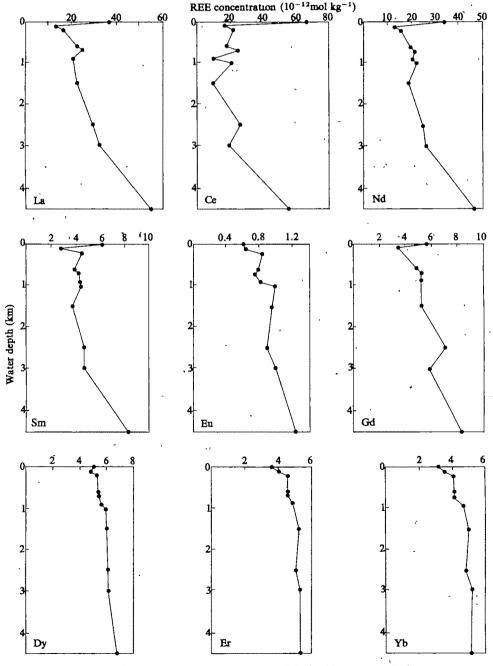


Fig. 1 Depth profiles of the REE at RRS Shackleton station 1B/79.

base of the mixed layer. In addition, the profiles of the light REE show surface maxima, that is, a surface-water enrichment:

The deep-water enrichment was suspected from earlier work¹. Some of the very early results are extremely high and must be regarded with suspicion. However, previous results^{7,8,24,25} show concentration levels similar to those reported here and include deep-water values which are higher than for shallow sample(s) from the same station. On the basis of this earlier work it has also been concluded¹ that REE levels in the deep Pacific are higher than those in the deep Atlantic. Modern data do not confirm this view. Indeed, a comparison of the most precise Nd data for the Atlantic (this study) and for the Pacific (the two analyses in refs^{7,8}) show that the Pacific values are ~30% lower at an equivalent water depth. Clearly, it is premature to make an assessment.

Table 1 also reveals that the deep-water enrichment varies in magnitude throughout the REE group in a systematic fashion. The proportional increase in concentration towards the bottom is greater for the light REE than for the heavy REE. For example, Yb-Dy are enriched by a factor of ~1.4 at 4.5-km depth relative to the base of the mixed layer (100 m) whereas Nd is enriched 3.6 times and La, 4.2 times. Like this deep-water behaviour, the surface-water enrichments of the REE vary with atomic number but with an important difference: the light REE are enriched (for example, La by a factor of 2.8 compared with the base of the mixed layer) whereas the heavy REE are not enriched or are slightly depleted. Because of their similarity, the surface-water and deep-water enrichments show a striking correlation (Fig. 2). Note that the enrichments of Ce and Eu are anomalous compared with values that may be predicted from interpolations between their respective REE neighbours. Both the fractionation of the light- from heavy-REE (or vice versa) at the surface and bottom boundaries of the water column and the anomalous behaviours of Ce and Eu are compatible with the chemistry of the REE group.

REE patterns

The shale-normalized REE pattern of seawater has been shown²⁴ to be characterized by a monotonic increase in the concentrations of the heavy REE relative to the light REE and by a marked depletion in Ce relative to its REE neighbours (that is, a negative cerium anomaly). The REE patterns obtained here may be placed in three groups (Fig. 3):

(1) The pattern for surface seawater is unlike any reported previously. The pattern is flat and shale-like with a slight negative Ce anomaly, but with a prominent negative Eu anomaly.

(2) Seawater below the mixed layer has a pattern similar to that reported previously although with a less striking Ce anomaly. With increasing depth the heavy REE enrichment becomes less significant, a negative Eu anomaly develops and the negative Ce anomaly strengthens and then weakens (Fig. 4). As a consequence, the pattern for the deepest sample

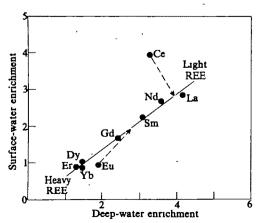


Fig. 2 Surface-water/deep-water enrichments of the REE relative to concentrations at base of the mixed layer.

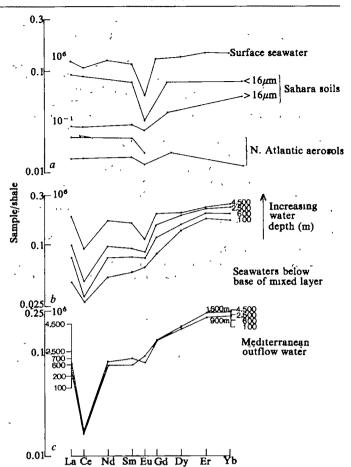


Fig. 3 REE patterns: a, surface seawater compared with Sahara soils and North Atlantic aerosols; b, seawaters below base of the mixed layer, 100-4,500 m; c, seawaters in the core of Mediterranean water, 900-1,500 m.

analysed begins to resemble that for surface seawater except for its Ce anomaly.

(3) The seawaters between \sim 900 and 1,500 m, representing Mediterranean overflow water, also show 'typical' seawater patterns but are anomalous relative to waters above and below in displaying stronger negative Ce anomalies, weaker negative Eu anomalies, and higher heavy REE and lower light REE concentrations (Fig. 3c) give a more marked heavy REE enrichment (Fig. 4).

Boundary layer processes

Because of the deep-water enrichment of the REE and the contention that REE are higher in the Pacific than the Atlantic, the REE have been likened to nitrate and phosphate and to those trace elements whose depletion in surface waters has been interpreted in terms of biological removal^{1,7,8}. From the present results it seems unlikely that the REE exhibit simple nutrient-like behaviour in seawater. Overall, the REE profiles are dissimilar to the profiles both of phosphate (which reflects near-surface regeneration of soft tissues) and of silicate (which reflects deep-water regeneration of hard skeletal materials) except that all increase with depth (Figs 1, 4). Therefore, one hypothesis is that the behaviour of the REE in waters below the mixed layer is nutrient-like, the light REE following Si and the heavy REE following P. However, the surface-water behaviour of the REE is not nutrient-like. Both silicate and phosphate show near-zero concentrations in surface waters unlike the REE. The light REE also show a significant surface maximum.

The profiles for the light REE are reminiscent of those of Cu (ref. 18) Al (ref. 21) which have been interpreted in terms of inputs across the surface and bottom boundaries of the oceans coupled with scavenging by a combination of biological removal

and general (inorganic?) removal with subsequent incorporation in and removal by zooplanktonic faecal matter or uptake on surfaces of skeletal fragments. For the REE, the association with colloidal matter such as iron-oxide flocs inside tests, or as coatings, or in biogenic particles has been favoured 10. Although we cannot yet identify any specific carrier for the REE we can show from the general depth trends of the Ree and of P, Si and alkalinity (Table 2a) that (1) planktonic material contains insufficient REE to account for the observed deep-water enrichments of the light REE (little data are available for the heavy REE); (2) REE in foraminiferal carbonate can just account for the heavy REE enrichments but not for the light REE: (3) diatom opal can readily account for all the enrichments. REE/P/Si/Ca ratios in large particles from sediment traps suggest that such material contains a large excess of REE compared with the levels needed to satisfy the deep-water enrichments. Hence, we propose that the REE are removed selectively from solution in surface waters by scavenging, the light REE being removed more effectively than the heavy REE. This process is consistent with the previously observed REE pattern for seawater which led Goldberg²⁴ to suggest that the heavy REE enrichment may be attributed either to the increasing stability of complexes of the heavier REE with seawater ligands or to their differential adsorption on solid phases. Our observations clearly indicate that Ce behaves differently from the other REE. The fractionation of the light and heavy 3+ REE is at its maximum at the base of the mixed layer (the same is true for the least negative Eu anomaly) showing that the surface removal processes are restricted to this zone (Fig. 4). In contrast, the negative Ce anomaly develops over a depth of 500 m or more, pointing to a different process, presumably the oxidation of Ce³⁺ to the less soluble 4+ valency state.

As well as REE removal in surface waters there is tentative evidence for deep-water removal. Below ~2 km where the potential temperature-salinity relationship is linear, plots of the REE against either of these conservative tracers show negative curvature, indicating removal of the REE in deep waters. However, the deep circulation of the Atlantic is complex and deep-water scavenging is not necessarily implied by this observation.

What are the causes of inputs at the surface and bottom boundary layers? Analogy with Cu and Al suggests that the bottom source is the interstitial waters of marine sediments into which the REE are released during early diagenesis when the REE-containing phases dissolve or lose their binding capacities. Again, the difficulty of modelling chemical gradients in Atlantic deep waters means that no other benthic flux need be postulated other than from regeneration. However, a sediment source is consistent with our work on oceanic ferromanganese nodules and sediments which indicates that the REE are mobile during early diagenesis ^{6,10,11}. The diffusive benthic fluxes which would

be required to maintain this deep-water enrichment are considerable—there must be strong REE gradients in the interstitial waters. Minimum benthic fluxes calculated using residence times of 100–400 yr for North Atlantic deep water are comparable in magnitude to the average rates of REE accumulation in deep-sea sediments^{1,27}. Hence, if diffusion from sediments is an important source, the uppermost layer of deep sea sediments should be strongly enriched in REE, as indeed seems to be the case for La (ref. 28). Because the cycling of the REE through the surface layer of sediments seems to be such an important part of the oceanic REE cycle it is obvious why ferromanganese nodules and coexisting sediments have the same Nd isotopic composition as deep ocean water^{7,8,10,12}. Nodules obtain Nd through sediment diagenesis which also provides the Nd in bottom waters which itself is scavenged by sedimenting particles.

The source of the surface-water enrichment relative to the bottom of the mixed layer in light REE is probably aeolian. The other trace elements for which surface-water enrichment has been observed are Cu, Mn, Pb and Al (refs 18–21). Analogy with atmospheric input tracers ²¹⁰Pb and ⁷Be which also show surface enrichment suggests that the inputs are aeolian. For the REE it is possible to employ the 'internal' tracer of the REE pattern for comparison with potential input material. This exercise (Fig. 3a) shows that the striking REE pattern for surface seawater at our station is comparable with that for Sahara Desert soils and for the North Atlantic (Sahara) marine aerosol²⁹. Nd isotope studies on the water and aerosols would be useful to confirm the interpretation but atmospheric input is probably the major source of the REE in North Atlantic surface water in this region.

Hydrographical signals

We cannot rule out the possibility that certain parts of the vertical profiles may be primarily hydrographical signals. First, the surface values could reflect advected input of continental REE and the deep-water values could mean that North Atlantic deep and bottom water forms with very high REE contents. Second, and more plausible, the mid-water extrema in the REE profiles probably reflect a distinctly different composition of Mediterranean water compared with the Atlantic water masses. Clearly, water in the Mediterranean outflow, centred at ~1,200 m at this station (Fig. 4), has a distinctive REE pattern compared with adjacent waters (Fig. 3): that is, a larger heavy REE enrichment, a more negative Ce anomaly and a less negative Eu anomaly (Fig. 4). We found the 143Nd/144Nd ratio of biogenic carbonate from the Mediterranean to be $0.512275 \pm$ 20, slightly higher than for Atlantic ratios^{7,8}. Hence, the use of REE patterns and Nd isotopic ratios as modern and palaeooceanographic tracers of water masses and ocean basins seems feasible.

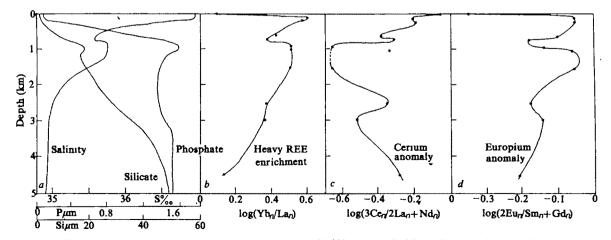


Fig. 4 Depth profiles for hydrographic and nutrient features for station 1B/79 compared with profiles of shale-normalized REE parameters:

a, salinity, phosphate and silicate; b, heavy REE enrichment; c, cerium anomaly; d, europium anomaly.

Table 2 Enrichment ratios and residence times of the REE									•	
a, Comparison of REE enrichment ratios for possible carriers with elemental compositions										
a, Comparison of REE enrichment ratios for possiti	La La	Ce	Nd	Sm	Eu	Gd	Dy	Er	Yb	
		~		OM.	Du		Σ,		70	
Plankton	20	26	20	2.0	0.20	2.2	1.4	1.0	1.4	
$\Delta REE/\Delta P$ in water column (mol ratio $\times 10^6$)	28	26	22	3.8	0.39	3.3	1.4	1.2	1.4	
REE content calculated from ratio*/measured content†	19	10	70	-	***	-	19	-	-	
· _ · · · · · · · · · · · · · ·	3-19									
10 ³ ratio/(REE/P) in sediment trap and faecal materials‡	3-19									
Opal										
$\Delta REE/\Delta Si$ in water column (mol ratio $\times 10^6$)	0.86	0.79	0.68	0.12	0.012	0.10	0.042	0.035	0.042	
REE content calculated from ratios/measured	0.14	0.090	0.098	0.12	0.012	0.062	0.027	0.039	0.056	
content†	0.14	0.070	0.076	0,077	0.050	0.002	0.027	0.037	0.050	
10 ³ ratio/(REE/Si) in sediment trap and faecal	18-21									
materials‡	10 21									
Carbonate										
ΔREE/0.5Δalkali in water column (mol ratio×	4.1	3.8	3.3	0.56	0.058	0.49	0.21	0.17	0.20	
106)		2.0		****	3.020	٠,,,	0.22	,	0.20	
REE content calculated from ratio /measured	4.5	15	4.8	4.2	1.5	2.7	1.0	1.0	1.2	
content†										
10 ³ ratio/(REE/Ca) in sediment trap and faecal	22-164									
materials‡										
b. Residence times of the REE										
Water column standing crop (10 ⁻⁹ mol cm ⁻²)¶	14	13	13	2.4	0.44	2.8	2.7	2.3	2.1	
Residence time in surface ocean relative to	14	13	13	2.4	0.44	2.0	2.1	4.3	2.1	
aeolian input (yr)#										
. Maximum	6.2	4.4	6.6	7.8	19	11	17	20	20	
Minimum	0.2	0.87	1.0	0.93	1.1	1.1	1.2	1.3	1.2	
Overall oceanic residence time (yr)	0.71	0.07	1.0	0.55	1.1	1.1	1.4	1.5	1.2	
Relative to river input**	1,500	720	1,100	1,100	910	1,200	1,400	2,000	2,200	
From acolian + river input † †	240	110	450	220	470	300	340	420	410	

^{*}Assuming molecular weight of plankton = 1,527 ($C_{106}N_{16}P_1$); † from ref. 10; ‡ from ref. 37; § assuming (as in ref. 22) molecular weight of opal = 150.1 (SiO₂.5H₂O); ¶ assuming Δ Ca²⁺ = 0.5 Δ alkalinity; ¶ integrated areas in Fig. 1; # see text for methods of calculation; ** standing crop×oceanic area of $361 \times 10^{16} \text{cm}^2/\text{REE}$ in river water (based on ref. 9)×global river discharge rate of $4 \times 10^{19} \text{cm}^3 \text{ yr}^{-1}$. Values not corrected for REE loss in estuaries which would increase τ by factor of ~4; †† assumes La flux of 60×10^{-12} mol cm⁻² yr⁻¹ (50 aeolian—see text; 10 rivers) and other REE pro rata. Probably minimum values of τ because of relatively large aeolian flux in north-east Atlantic; ‡‡ using Piper's estimates (ref. 1) of average REE accumulation rates in sediments.

690

400

620

600

460

The oceanic REE cycle

From sediment output##

From the above discussion we can summarize the geochemical cycle of the REE at our North Atlantic station and compute their oceanic residence times. As a first approximation we will assume a steady state and ignore horizontal transport. The major surface input appears to be atmospheric and the REE are leached from aeolian particles without significant fractionation (except possibly for Ce and Eu). The dissolved REE are scavenged from surface waters with preferential removal of the light REE. Thus, the residence time (τ) of La in the surface ocean must be less than that of Yb, the upper limit for the least reactive heavy REE being the residence time of the mixed layer $(\tau_{\rm H_2O})$ of ~20 yr (ref. 30). For typical particulate loadings in surface seawater near station 1B/79 of ~30 µg per kg (refs 31, 32) and for the enrichment of double crustal abundance of the REE in marine aerosols²⁹, there would be $\sim 7.5 \times 10^{-12}$ mol kg⁻¹ of particulate La in surface seawater if no leaching occurs at all, compared with $\sim 37 \times 10^{-12}$ mol kg⁻¹ of dissolved La. Therefore τ_{La} in surface water must be 37/7.5 times the residence time of unreactive particles ($\sim 0.2 \text{ yr}^{33}$) that is $\sim 1 \text{ yr}$, if all the REE are leached from the aeolian input. This is a minimum value for the residence time because if, say, only 15% dissolves then $\tau_{La} \sim 6$ yr. Because τ_{La} must be $<\tau_{H2O}$ then at least $\sim 5\%$ of the REE must be leached.

Another approach to estimating τ is from the REE fractionation below, relative to in, the mixed layer. As the Yb/La ratio decreases by a factor of 3.2, the upper limit for $\tau_{\rm La}$ in the mixed layer is about one-third of $\tau_{\rm H2O}$, that is 6.2 yr. Note the anomalous positions of Ce and Eu in the residence times computed in these ways (Table 2b). $\tau_{\rm Ce}$ is shorter and $\tau_{\rm Bu}$ is longer than their position in the REE group might imply.

These estimates for surface ocean residence times for the REE are compatible with aerosol data. From τ and the amount of a rare earth element present in the mixed layer (a) the input flux can be predicted from a/τ . The figure for La is $58\times 10^{-12}\,\mathrm{mol\,cm^{-2}\,yr^{-1}}$ (using τ_{max}). In comparison, typical Al deposition rates $^{33.34}$ of $\sim 5\,\mu\mathrm{g\,cm^{-2}\,yr^{-1}}$ together with La/Al in aerosols which average 14×10^{-4} by weight (using data in ref. 29; La and Al seem to correlate in marine particulates) gives a similar flux of $50\times 10^{-12}\,\mathrm{mol\,cm^{-2}\,yr^{-1}}$. Alternatively, La in the North Atlantic aerosol has been measured $^{24.35}$ at 0.28 ng m $^{-3}$ (using typical Al in aerosols $^{33-35}$ of 150 ng m $^{-3}$ and the above La/Al ratio gives a similar value, 0.21 ng m $^{-3}$). Using a typical depositional velocity of $1\,\mathrm{cm\,s^{-1}}$ gives $64\times 10^{-12}\,\mathrm{mol\,cm^{-2}\,yr^{-1}}$.

720

810

980

970

The rapid transport to the deep ocean of the REE scavenged in the surface ocean and their release from the sediment explains the similar REE patterns of deep and surface waters and the link in their enrichments shown in Fig. 2. Thus, the surfacewater enrichments relative to 100 m depth (Fig. 1) of the light REE reflects their preferential removal by scavenging, the sedimented products of which release the REE during early diagenesis leading to stronger deep-water gradients for the light than for the heavy REE. However, we cannot yet rule out in situ regeneration as a source of the REE in deep waters. The Ce anomaly in Fig. 2 might be expected from its redox chemistry in surface seawaters and sediments. In surface waters, Ce released from particles will oxidize and so be removed relative to the 3+ REE. In deep-sea sediments this same reaction means that Ce released from decomposing particles will be less mobile than the 3+ REE (this is observed in terms of the positive Ce anomaly in most oceanic ferromanganese nodules¹) leading to a relatively weaker deep-water gradient for Ce.

The surface and deep sources for the REE suggested here can explain the imbalance in the overall REE budget recognized by Piper¹ and Martin³⁶ who attempted to balance sediment removal with river input only. The residence times with respect to river input (Table 2b—these are minimum values as they are uncorrected for REE removal which occurs in at least some estuaries^{9,36}) are much too long to represent the total input in view of the water mass effects described earlier. A major atmospheric input will redress this balance (Table 2b). Overall, accurate values for τ are difficult to estimate because the atmospheric fluxes into the north-east Atlantic are probably much larger than the oceanic average. However, the atmospheric fluxes may be highly significant. Equally, a benthic flux of the magnitude of the river flux would allow the long riverine residence times to be compatible with the short scavenging residence times. Thus, overall, the REE may reside in the ocean for ~500 yr before being scavenged but are regenerated on average, say, five times before being finally buried and therefore would have residence times with respect to river input of 2,500 yr. The computed values for the overall residence times of the REE (Table 2b) being less than the oceanic mixing time are compatible with the tracing of water masses using REE contents as suggested by the Mediterranean water. In particular, τ for the light REE are short. Thus, the difference between water masses should be more pronounced for the light REE than for the heavy REE, as is observed (Fig. 2). In addition, the value for $\tau_{\rm Nd}$ is consistent with the inter-oceanic differences in the $^{143}{\rm Nd}/^{144}{\rm Nd}$ ratio of seawater $^{7.8,12}$. Although the above described residence times have various shortcomings and uncertainties due to the limited data base and inherent assumptions, their trends clearly reflect our suggested input and removal mechanisms and our basic conclusion that the REE are rapidly cycled in the marine environment.

We thank members of RRS Shackleton cruise 1B/79 and oceanographic and isotopic colleagues at Leeds for their help; and W. & T. Avery Ltd for the use of scales to weigh the ~1,000 kg of seawater. This research was supported by NERC grants GR3/3125 and GR3/4444.

Received 21 October 1981, accepted 21 January 1982

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Genome instability in a region of human DNA enriched in Alu repeat sequences

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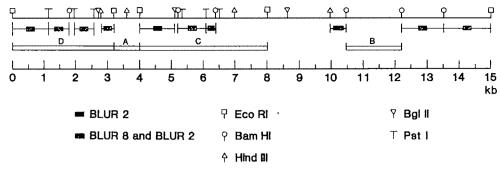
DNA rearrangements involving restriction fragment length polymorphism and variation in copy number were detected in the human genome by blot hybridization with a cloned segment of human DNA initially present in a cluster of Alu repeat sequences. These rearrangements involve both extrachromosomal circular duplex DNAs and integrated sequences, indicating the presence of transposable elements in human cells.

RENATURATION kinetics¹ and genomic library screening² have shown that the human genome contains at least 400,000 copies of the 300-base pair (bp) Alu repeat sequence, the predominant repetitive sequence in man^{3,4}. Members of the Alu repeat family have been proposed to have cellular functions on the basis of various characteristics, including the widely dispersed arrangement of Alu repeats, the conservation of a 40-bp sequence of the Alu repeat in mammalian evolution, the homology of a 14-bp segment with a sequence near the replication origin in several DNA viruses, and the observation that the Alu repeat is represented in transcripts generated by both

RNA polymerase II and III^{5,6}. Thus, a role for Alu repeat sequences in the initiation of DNA replication and nuclear RNA processing has been postulated5,6

The most striking feature of the Alu repeat family is its large numerical representation in the human genome, which suggests that Alu repeat sequences might be involved in genetic rearrangements, a role which could be identified if we consider the human genome to be a dynamic structure. Although most members of the Alu family are scattered throughout the human genome, some may be clustered in certain genomic regions. Such an arrangement would provide a good opportunity to test

Fig. 1 Localization of Alu repeat sequences within the restriction enzyme cleavage map of AH15, AH15 was isolated from a human genomic library as a clone hybridizing to total nick-translated repetitive sequences, grown as previously described2, and the human insert was characterized with respect to the distribution of repetitive sequences. The orientation of the human insert with respect to the left and right arms of the vector was facilitated by knowing the restriction map for Charon 4A24 The localization of Alu repeat sequences was initially derived for the restriction map of AH15, and confirmed for



the restriction map of several segments of λ H15 (subcloned in to pBR322 or pACYC184 and indicated by segments D, A, C and B, respectively) using Southern blot hybridization. with nick-translated Alu repeat inserts from either the clone BLUR 8 or BLUR 2 (ref. 4) as previously described.

the hypothesis that repetitive sequences facilitate genetic rearrangements.

Alu repeat sequences are clustered in regions of the human genome

We searched a human genomic DNA library⁷ for clones containing several Alu repeat sequences, having previously shown that more than 95% of this library contained members of the Alu repeat family² and that in several clones, two or more copies were present (unpublished observation). Clone λ H15, randomly selected as strongly hybridizing to repetitive sequences, was examined in detail. An initial digestion of this clone with several restriction enzymes, followed by Southern blot hybridization with the cloned Alu repeat sequence probes BLUR 2 and BLUR 8 (ref. 4), gave several hybridizing bands, indicating that many Alu repeat family members were present in this 15-kilobase (kb) DNA insert. Double digestion with restriction

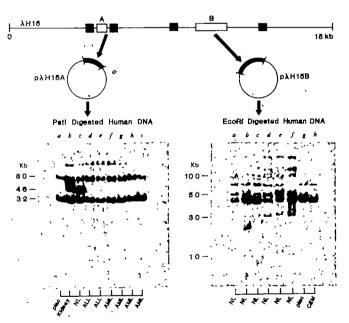


Fig. 2 Experimental design used to investigate DNA rearrangements in tissues of normal and leukaemic individuals. The dark boxes on the human insert of \(\lambda\text{H15}\) represent \(Alu\) repeats sequences flanking the DNA fragments subcloned in pACYC184 (pAH15A) and pBR322 (pAH15B). Subclones were prepared as previously described. Human DNA (10 \(\mu\)g) prepared as described elsewhere \$^{26}\$ was digested with an excess of \$Psil\$ (left side) or \$EcoRI\$ (right side) for 2 h and electrophoresed through 1% agarose gels in 50 mM Tris (pH 8.2), 50 mM boric acid, 10 mM EDTA at 80 V for 6 h. After electrophoresis, the DNA was transferred to nitrocellulose by the method of Southern \$^{25}\$ and the resulting blots were hybridized with 2 \times 10^7\$ c.p.m of labelled pAH15A or pAH15B DNA, prepared by nick translation \$^{27}\$ The hybridization was performed in the presence of 10% dextran sulphate \$^{26}\$ as previously described \$^{26}\$. Filters were exposed for 12 h to X-ray films with intensifier screens. The approximate sizes of DNA in kilobase pairs (kb) in the bands are indicated on the left. plac, Placenta, NL, normal leukocytes; AML, acute myeologenous leukaemia; ALL, acute lymphocytic leukaemia; CEM, a human lymphoblastoid cell line Each lane contains DNA from a different individual.

endonucleases *Eco*RI plus *Bam*HI and *Eco*RI plus *Pst*I showed 7 and 12 bands, respectively, hybridizing to these cloned members of the *Alu* repeat family.

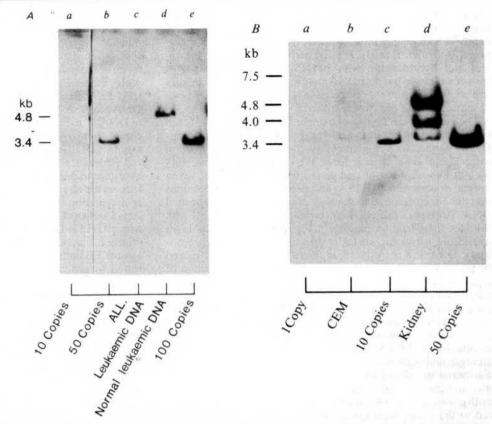
Figure 1 shows the restriction enzyme cleavage map of λ H15, together with the approximate locations of the Alu repeat family members. At least 10 Alu repeat sequences are clustered in this clone, a minimal estimate due to the high degree of divergence among different members of the Alu repeat family⁴. Two observations support this assertion. First, we identified an Alu repeat sequence hybridizing very strongly to BLUR 2 (dark box in Fig. 1) and very weakly to BLUR 8. The sequence divergence between BLUR 8 and BLUR 2 is 24%, thus precluding cross-hybridization in the conditions used. This observation suggests that some members of the Alu repeat family have diverged considerably in the Alu repeat cluster of λH15. Second, in R-loop hybridization between poly(A)⁺ RNA, derived from a human lymphoblastoid line and subclone phH15C (Fig. 1), two additional Alu-like sequences have been detected and positioned with respect to the Alu sequence distribution derived by Southern blot analysis⁶. We may therefore consider the segment of $\lambda H15$ between the first and the fourth EcoRI sites (Fig. 1) as a cluster of Alu repeat sequences.

A role for Alu repeat sequences in genomic rearrangements

The unusual structure of this region provides an opportunity to test the hypothesis that Alu repeat clustering may be involved in genome rearrangements. To investigate this possibility we took advantage of the observation that in λ H15, DNA segments of single copy or very low repetition frequency are flanked on both sides by Alu repeats. Subclones of two of these regions (A and B in Fig. 1) were constructed for use as hybridization probes to detect restriction fragment length polymorphisms (RFLPs) in the genomic region in which the human insert of λH15 is localized. If DNA rearrangements occur in this region at a relatively high frequency, they should be detectable by RFLP analysis. We therefore used cleavage with restriction enzymes and blot hybridization experiments to examine the DNA from tissues of healthy individuals and patients with neoplastic haematological diseases, and found substantial polymorphism with DNA from tissues of healthy individuals using both A and B region probes (Fig. 2). Digestion of several of the DNA samples with other restriction enzymes also revealed patterns of polymorphism. Furthermore, we found that the intensity of one hybridizing band differed markedly among DNAs derived from leukocytes of a normal donor (Fig. 2, lane c, left side) and DNA derived from leukaemic leukocytes (Fig. 2, lanes d-i). These results indicate both variation in the number of hybridizing sequences (copy number) and multiple forms revealed by RFLP of the sequence in human DNA that hybridizes to pλH15A.

Using a gene copy number reconstitution experiment, we determined and compared the number of sequences hybridizing to $p\lambda H15A$ in DNAs derived from leukocytes of a patient with acute lymphocytic leukaemia and a normal individual. The method used has sufficient sensitivity and reproducibility to

Fig. 3 A, determination of the number of human genomic DNA sequences complementary to pAH15A. The numof sequences hybridizing to pλH15A was determined as follows: 5 μg of DNA derived from leukaemic lymphocytes of a patient with acute lymphocytic leukaemia (ALL) (lane c) and from peripheral white blood cells of a normal individual (lane d) digested with BamHI and electrophoresed on a 1% agarose gel with various amounts of BamHI-digested AH15 DNA from which pAH15A was derived. The copy number reference, BamHI-digested AH15, was applied in amounts corresponding to 10, 50 and 100 copies per haploid genome in 5 ug of human DNA (lanes a, b and e respectively). These lanes also contained 5 µg of EcoRI-digested chicken DNA used as carrier. Assuming a human haploid genome size of 3×10^9 bp, the 45-kb λH15 constitutes 1.53×10⁻⁵ of the human haploid genome. Therefore, the amount of AH15 in 5 µg of human DNA, if present at one copy per haploid genome, is $7.6\times10^{-5}\,\mu g$. To apply 10, 50 and 100 copies, 4.5 μg of $\lambda H15$ DNA were digested with BamHI, and the reaction was stopped by adding onefifth volume of a solution containing 50% glycerol, 1% SDS and 0.5% bromophenol blue. Appropriate dilutions were made from this solution. B, number of genomic sequences complementary to pλH15A in DNA of a human lymphoblastoid cell line (CEM) and normal kidney. DNA isolated from



CEM cells and kidney was electrophoresed with 1, 10 and 50 copies of BamHI-digested λ H15 DNA used as an internal standard, and then blot-hybridized to p λ H15A as described earlier. Densitometric scans of the autoradiograms demonstrate that the band at 3.4 kb hybridizing to p λ H15A in CEM cells corresponds to three copies in CEM cells and to approximately six copies in kidney tissue.

give reliable estimates of gene copy numbers 8,9,10 . Titration experiments (Fig. 3A) show that more than 50 copies of sequences hybridizing to $p\lambda H15A$ are present in the leukocytes of the normal individual, compared with less than 5 copies in leukaemic leukocytes. In all the patients with acute leukaemia so far examined (10 cases) we found less than five copies of $p\lambda H15A$ -hybridizing sequences. In contrast, of six patients with chronic myelogenous leukaemia examined, four showed an autoradiographic density comparable with that of healthy individuals, whereas two had copy numbers comparable with that found in acute lymphocytic leukaemia.

The number of sequences hybridizing to pλH15A in DNA derived from leukocytes of normal donors was rather variable but was usually more than 50 copies per haploid genome equivalent. In control experiments, the DNAs derived from normal and leukaemic leukocytes were hybridized with a recombinant plasmid containing the coding region of the human placental lactogen (HPL) gene, present in three to four copies per haploid genome ¹⁰, and equal intensities of hybridizing bands were observed in both DNA groups. We can therefore exclude variations in hybridization efficiency as an explanation of the differences in copy number observed using the pλH15A DNA as probe.

Figure 3B shows an additional example of variations in copy number of sequences hybridizing with p λ H15A. A gene copy number reconstitution experiment reveals that in DNA derived from either a normal kidney tissue or a human lymphoblastoid cell line, a meaningful variation exists in the content of sequences hybridizing to p λ H15A. The bands at 4.8 and 4.0 kb observed in kidney DNA (Fig. 3B, lane d) are not detectable at all in CEM DNA (Fig. 3B, lane b). The only band in common with these DNAs has a size of 3.4 kb and is present at three copies in the lymphoblastoid cell line and six copies in the kidney tissue per haploid genome. The 3.4-kb band, common to the human DNAs shown in Fig. 3, corresponds to the region of clone λ H15 between the BamHI sites at 1.8 and 5.2 kb shown in Fig. 1. We therefore identified variations in copy

number of a specific genomic sequence of human DNA and the results described below indicate that some of the hybridizing bands correspond to circular extrachromosomal molecules.

DNA rearrangements exist in different tissues of the same individuals

Having found variations in copy number and RFLPs in this region of the genome, we next investigated whether different forms of the hybridizing sequence occurred within different tissues of the same individual. A surprising result was that when DNA isolated from a 22-yr old woman who had died of acute lymphocytic leukaemia was digested with EcoRI and hybridized with p\u00bbH15A (Fig. 4A), both RFLPs and a marked difference in the copy number of sequences hybridizing to phH15A were detected among DNAs derived from liver, lung, brain and other organs. Some caution is necessary in interpreting the observed pattern of RFLP because this patient had received combination chemotherapy of doxorubicin, vincristine, cytosine arabinoside and prednisone for 4 days. Nevertheless, we observed RFLPs in these tissues following digestion with EcoRI, BamHI and HindIII which further indicated that tissue-related DNA rearrangements had occurred. A gene copy number reconstitution experiment showed that more than 50 copies of a DNA sequence hybridizing to pAH15A are present in lung and brain tissues, whereas less than 10 copies are present in spleen and kidney tissue (data now shown). Finally, RFLP was also detected in the DNA derived from different organs of a 40-yr old female who had died of melanoma (Fig. 4B), again indicating a frequent occurrence of tissue-related sequence rearrangement using the p\u00e4H15A probe.

Certain sequences hybridizing to p\(\text{H15A} \) occur as extrachromosomal molecules

Variations in the copy number of sequences hybridizing to pλH15A may indicate that these sequences occur in extra-

chromosomal form and are differentially amplified in different tissues or physiological states. Initially, we observed a major band \sim 4.8 kb long in most of the DNAs examined after digestion with EcoRI, BamHI, HindIII and Bg/II (data now shown) and blot hybridization with $p\lambda H15A$. Digestion with PsII revealed a single band of DNA that was \sim 150–200 bp smaller and probably represents cleavage in a single region where two or more PsII sites are closely spaced.

The very unusual finding of a single 4.8-kb fragment for each of the restriction enzymes again suggested that molecules containing sequences complementary to phH15A may exist in extrachromosomal circular form. We examined this possibility by blot hybridization of undigested leukocyte DNA of seven normal donors with phH15A, and detected bands in positions that did not correspond to the bulk of undigested chromosomal DNA. (Compare the ethidium bromide staining pattern with the blot hybridization pattern in Fig. 5A.) The predominant hybridizing bands migrate faster than the bulk of linear chromosomal DNA and correspond to closed circular (A) and nicked circular (B) forms of duplex DNA. A lower concentration of linear duplex DNA of this molecular weight is detected as a faint band migrating slightly faster than nicked circular DNA (labelled 4.8 kb in Fig. 5A, B). These three hybridizing bands occupy relative positions in the gel that would correspond to the positions of nicked circular, linear and covalently closed circular forms of 4.8-kb duplex DNA in similar conditions (ionic strength and voltage gradient) of agarose gel electrophoresis¹ Furthermore, when leukocyte DNA from a normal individual was subjected to a time course digestion with BamHI (Fig. 5B). both closed circular (A) and nicked circular forms were converted to the linear duplex form (labelled 4.8 kb). Note that the nicked circular and linear duplex forms of this DNA species are in low concentration in the undigested sample (Fig. 5B, first lane from the right).

Additional discrete bands of DNA hybridizing with p\(\text{H15A}\) were detected (Fig. 5A). One of them seems to be at an intermediate position between the closed circular and nicked circular forms of the predominant bands of hybridizing DNA, while the remaining bands seem to migrate more slowly than the nicked circular form of the predominant species (bands above B). These additional bands may represent closed circular, linear and nicked circular duplex forms, respectively, of at least

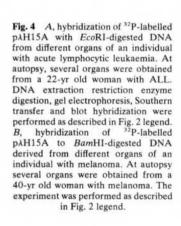
two other species of extrachromosomal DNA that contain sequences complementary to $p\lambda H15A$ but that are present at much lower copy numbers. Perhaps one, but not both, of these species represents a circular oligomer, possibly a circular dimer form, of the predominant species (A and B in Fig. 5A). Furthermore, the time course of digestion of these DNAs with BamHI (Fig. 5B) converts all but one of the higher molecular weight DNA bands to 4.8-kb linear duplex DNA. This remaining band of DNA (C in Fig. 5B), therefore, must represent one additional closed circular DNA species containing sequences complementary to $p\lambda H15A$.

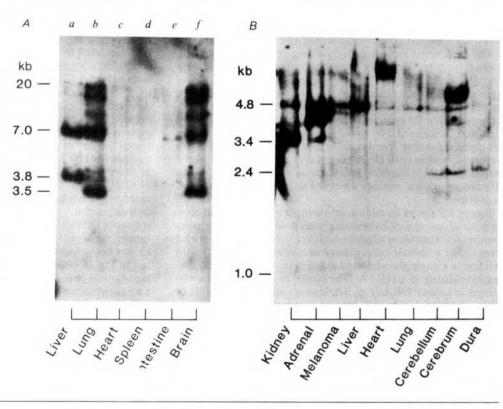
To visualize circular molecules containing sequences that hybridize to $p\lambda H15A$, the undigested chromosomal DNA from leukocytes of a normal individual was fractionated by velocity sedimentation through a sucrose gradient. Fractions were collected and an aliquot of each fraction was blotted and hybridized with $p\lambda H15A$ DNA. One fraction enriched in nicked circular molecules hybridizing to $p\lambda H15A$ was extensively dialysed against TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), concentrated to a final volume of 50 μ l and examined by electron microscopy using the aqueous Kleinschmidt technique¹².

As Fig. 5C shows, very low frequencies of circular duplex molecules were detected. Of 44 circular duplex molecules examined in this random sampling, 27% comprised a relatively uniform size class (upper row in Fig. 5C), with an average length of 4.71 ± 0.25 kb. A second, smaller homogeneous species was also detected (59% of all molecules), having an average length of 1.77 ± 0.35 kb. The remainder of circular duplex molecules appeared to be heterogeneous in size, with lengths ranging over 0.9-8.3 kb (lower row in Fig. 5C). The finding of a class of circular duplex molecules with a length of 4.7 kb is expected based on the results of blot hybridization experiments with pAH15, which had indicated the existence of a 4.8-kb circular duplex molecule. Supercoiled molecules (not shown) were detected in a faster sedimenting fraction of the sucrose gradient using the formamide modification¹² of the Kleinschmidt technique for electron microscopy.

Discussion

It is remarkable that Alu repeat sequences can exist in a clustered arrangement in the human genome, as revealed by the detailed analyses of the recombinant clone $\lambda H15$. The





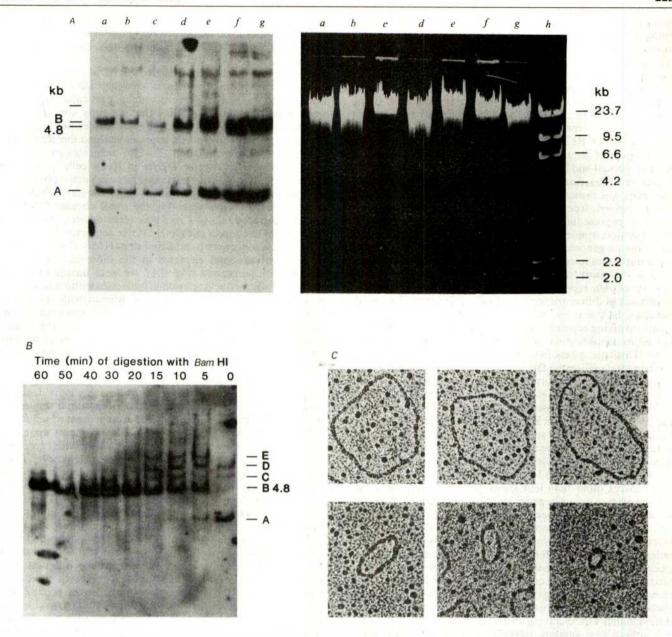


Fig. 5 A, hybridization of ³²P-labelled pλH15A to undigested DNA derived from leukocytes of normal donors. Undigested DNA (5 μg) from seven normal donors was electrophoresed through 1% agarose gels for ~15 h at 40 V. Southern transfer and blot hybridization were performed as described earlier. Hybridization of ³²P-labelled 0.8-kb human insert, purified by two cycles of gel electrophoresis, gave the same bands and relative intensities as blotting with the entire plasmid. B, time course of BamHI digestion of normal leukocyte DNA and hybridization with ³²P-labelled pλH15A. DNA (40 μg) was digested with BamHI at 1 U per μg and 5-μg aliquots were removed at different times. Gel electrophoresis, Southern transfer and blot hybridization with pλH15A were performed as described previously. C, electron micrographs of nicked circular duplex DNAs detected at very low frequency in aqueous Kleinschmidt spreads of nuclear DNA. Undigested DNA (100 μg) from leukocytes of a normal donor was applied to a 10-40% sucrose gradient buffered with 1 mM NaCl, 10 mM Tris-HCl pH 8.0 and 10 mM EDTA, and centrifuged in a VTi 50 rotor at 50,000 r.p.m. for 3 h. Thirty-six fractions of 1.1 ml each were collected and 100 μl of each fraction were electrophoresed in a 1% agarose gel, transferred to a nitrocellulose filter and hybridized with pλH15A as described earlier. The fraction enriched in molecules hybridizing to pλH15A was dialysed extensively against TE buffer, concentrated to a final volume of 50 μl and spread for electron microscopy using the aqueous Kleinschmidt technique 12. The molecules presented in the upper row have an apparent homogeneous length of 4.7 kb and represent ~27% of all circular duplexes detected. Other circular duplexes detected were smaller and heterogeneous in size, as shown in the lower row of micrographs, except for one species having an average length of 1.77±0.35 kb. Length measurements were made relative to the bacteriophage PM2 DNA with a length of 10,260 bp. Scale bar, 1 μm.

clustered arrangement of Alu repeat sequences in $\lambda H15$ may also be common to other regions of the human genome. We have screened the human genomic library with $p\lambda H15A$, which contains a subcloned fragment of $\lambda H15$, to detect DNA regions contiguous with the segment known as H15. Thus far, we have detected nine clones and have characterized five of them in detail. We observed that Alu repeat sequences are abundant in all five clones (T. Somasundaram $et\ al.$, unpublished) which reveals the existence of blocks of Alu sequences in the human genome. This fact is particularly intriguing with regard to the mechanism by which the pattern of interspersion observed for the bulk of Alu sequences is generated.

Sequence analysis showed that the Alu family sequence has

a dimeric structure with a duplication of a region conserved in mammalian evolution 13 . This region, 40 nucleotides long, is present once in the B1 family 14 of repetitive sequences in mouse, which is analogous to the Alu family in man. This internal duplication could have arisen if the initial expansion of Alu sequences occurred in a clustered arrangement like that presented here. We may then visualize the pattern of interspersion of Alu repeat family members as arising from the sequential excision of Alu sequences and their subsequent insertion elsewhere in the genome by mechanisms involving nonhomologous or parahomologous recombination as proposed by Anderson et al. 15 . The pattern of interspersion may have been fixed in evolution, with certain Alu repeat members having been

recruited for specific cellular functions, for example, in the initiation of DNA replication⁵ and as promoter sites for RNA polymerase III^{5,16}.

The discovery of a clustered arrangement of Alu repeat sequences provided an opportunity to explore the general question of sequence rearrangements in the human genome, especially those that may involve Alu repeat sequences. Using sequences from λ H15 DNA, we have detected two distinct aspects of DNA rearrangement involving either differential copy number or RFLPs. There were significant variations in copy number of sequences hybridizing to $p\lambda$ H15A among and between normal and leukaemic individuals. Furthermore, some of this variation could be tissue related, as the same set of sequences was present at different concentrations in different tissues derived from a single individual (Fig. 4A). Thus, sequences represented in the fragments hybridizing to $p\lambda$ H15A are not subject to selective pressures that would fix their number in the human genome.

It is important to emphasize that the 0.8-kb EcoRI fragment, which is the insert fragment in p λ H15A, is itself embedded in a cluster of Alu repeat sequences in the human genome. The occurrence of differential copy number of this sequence could, therefore, be due to the increased frequency of recombination events involving repetitive sequences as proposed by Smith¹⁷ as well as amplification of extrachromosomal circular DNA forms. This latter phenomenon was clearly detected.

When total undigested DNA was electrophoresed and probed with $p\lambda H15A$, we were surprised to find three hybridizing bands that resembled the gel electrophoretic separation of nicked circular, linear and closed-circular duplex forms of DNA (Fig. 5A). Circular duplex DNAs were also detected by electron microscopy after velocity sedimentation of total DNA, although there were two major species with lengths of ~4.7 and 1.8 kb respectively. Circular molecules with lengths other than 4.8 kb and hybridizing to p\(\text{H15A}\) were detected in DNAs derived from sources other than leukocytes. A human skin fibroblast cell line after serial passage for eight generations was found to contain an additional species ~7.5 kb long. The DNAs from liver, lung and brain tissues of one individual (Fig. 4A) all contained extrachromosomal sequences present as circular molecules with sizes different from the predominant circular species detected in normal leukocytes. In particular, electron microscopic examination of the covalently closed circular DNA from lung tissue using propidium diiodide-CsCl density gradient centrifugation revealed a major population (~60%) of the circular DNAs having an average length of 3.4 ± 0.2 kb. In Southern blot analysis, p\u00e4H15A hybridized to this closed circular DNA population after EcoRI digestion and revealed one major band at 3.5 kb, essentially the same as identified by electron microscopy (B.C. et al., unpublished). The DNA from kidney tissue of another individual contained additional hybridizing sequences corresponding to 4.0-kb circular duplex molecules (B.C. et al., unpublished). These variations in size could reflect different genome rearrangements in formation of these circular molecules and may possibly involve duplications or deletions of Alu repeat sequences.

Blot hybridization of undigested leukocyte DNA with a cloned Alu repeat sequence, BLUR 8, or human repetitive DNA revealed the presence of Alu repeat sequences in the leukocyte 4.8-kb circular DNA (B.C. et al., unpublished). In this case, we detected several discrete bands, one of which represented very low molecular weight DNA in the size range corresponding to the length of the Alu repeat sequence. This observation may indicate that Alu repeat sequences themselves occur as small circular duplex DNAs.

Small polydisperse circular DNAs have also been reported to occur at different concentrations in different organs of chicken embryos as well as in the chicken bursa at various stages of development¹⁸. It is possible that the difference in copy number of circular DNAs detected in the leukocyte DNA of normal and leukaemic individuals could reflect the different stages of differentiation of the leukocyte populations. Small

polydisperse circular DNAs were first described for HeLa cells but were found to be quite heterogeneous in size, with a mean length of 0.32 µm (refs 19, 20). Interestingly, the small polydisperse circular DNAs in HeLa cells were shown to be of cytoplasmic but not mitochondrial origin²⁰. It is plausible that low concentrations of small polydisperse circular DNAs exist in the nucleus and, in the tissues we examined, particular size classes were selectively amplified. Note that the copy number of the 4.8-kb circular DNA we describe (~50 copies per leukocyte haploid genome) corresponds approximately to the copy number of the entire population (50–200 molecules per cell) of small polydisperse circular DNAs in HeLa cells²⁰, implying selective amplification of a particular size class in the population of polydisperse DNA examined. The circular DNAs we detected more closely resemble the small circular DNAs in nuclei of cultured cells of *Drosophila melanogaster*, which have been shown to contain middle-repetitive sequences²¹.

Although sequences hybridizing to phH15A DNA occurred as extrachromosomal elements in the different tissues of a woman with leukaemia (Fig. 4A), we were unable to detect, unequivocally, extrachromosomal molecules without restriction enzyme digestion in tissues from a woman with melanoma (Fig. 4B). However, DNA from tissues of this individual contained a 4.8-kb hybridizing band (Fig. 4B) which in other cases corresponded to extrachromosomal DNA. The other discrete bands in the different tissues of this individual may indeed represent RFLP of chromosomal sequences, Therefore, the occurrence of H15A hybridizing restriction fragments of different lengths in different tissues of the same individual may involve either chromosomal or extrachromosomal sequences. Furthermore, the copy numbers and arrangement of sequences hybridizing to phH15A as chromosomal segments appear to differ in other tissues. In this regard, a 3.4-kb BamHI fragment (Fig. 3A, B) is present in three copies in leukocyte DNA whereas at least six copies are present in kidney tissue (Fig. 3B), indicating that a duplication involving this segment has occurred to different extents in the two cell types. The occurrence of such variation in copy number is further confirmed by the finding that nine clones contain sequences homologous to phH15A in a human genomic DNA library established from fetal liver tissue⁷. Five of these clones were analysed and shown to represent segments of the human genome different from H15. Furthermore, in the liver tissue from which the library was established, the arrangement of sequences probed with p\lambda H15A differs from that found in leukocyte DNA. This conclusion is drawn from the observation that possibly two chromosomal fragments in PstI-digested leukocyte DNA hybridize with pλH15A (Fig. 2) rather than five or more as expected.

These combined studies indicate that polymorphisms and variation in copy number of chromosomal and extrachromosomal DNA sequences hybridizing with pλH15A reflect the presence of transposable elements in human cells. The recent observation that *copia*, the well known transposable element in *Drosophila*, exists in extrachromosomal form in cultured cells²² adds significance to the present results. In general, the human genome seems to be a dynamic structure in which variations can be introduced by sequence rearrangement, certain of which can lead to the formation of circular duplex DNA molecules. This genetic plasticity is quite characteristic of transposable elements, and the consequent genome alterations are relevant to evolutionary changes, while the DNA rearrangements may be involved in human cancer²³.

We thank Dr R. Shmookler Reis for many helpful suggestions during this work, Nancy Yen and Debbie Brock for technical assistance, T. Maniatis for the human genomic DNA library, P. Deininger and C. Schmid for the Alu repeat plasmids, and Abby Maizel for providing CEM cells. This investigation was supported by NIH grants GM 23965, GM 27774, CA 20124, CA 16672 and CA 16527, and Robert A. Welch Foundation grants G-267 and G-841. H.A.B.-S. was supported by a scholarship from the Consejo Nacional de Ciencia y Tecnología (CONACYT) of the Mexican government.

Received 25 August 1981, accepted 25 January 1982.

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A new model for white dwarf supernovae

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We show here that carbon-oxygen white dwarfs in close binary systems are likely progenitors of neutron stars and Type I supernovae (SNI). This conclusion is supported by different effects associated with crystallization of the carbon-oxygen mixture at relatively low temperatures ($T \le 5 \times 10^7$ K) and high densities $(\rho > 5 \times 10^9 \text{ g cm}^{-3})$. In particular, the existence of a pronounced extectic in the phase diagram of carbon-oxygen mixtures induces carbon-oxygen separation, with oxygen settling at the star's centre and carbon being rejected to lowerdensity layers. This not only favours 'quiet' or non-explosive collapse, but it may also induce off-centre ignitions at variable depths. We discuss their possible correspondence with the 'fast' to 'slow' SN I range.

Carbon-oxygen white dwarfs in close binary systems are generally thought to be at the origin of nova outbursts¹ and their significance for SNI explosions has been suggested^{2,3}. Current models of SN I light curves^{4,5} involve explosive carbonoxygen burning with ejection of variable amounts (from 0.5 to $1.0\,M_{\odot}$) of ⁵⁶Ni. This burning is triggered in various ways by mass accretion from the companion in the binary system. Several mechanisms have been proposed: central carbon deflagration for accretion rates in excess of 4×10⁻⁸ M_☉ yr (ref. 6); helium detonation at the base of the accreted layer, for $1.10^{-9} M_{\odot} \text{ yr}^{-1} \le \dot{M} \le 4 \times 10^{-8} M_{\odot} \text{ yr}^{-1}$ (ref. 7); finally, central carbon deflagration or low-depth helium detonation again, for $\dot{M} \leq 1 \times 10^{-9} M_{\odot} \,\mathrm{yr}^{-1}$, the former for white dwarf masses in excess of $1.2 M_{\odot}$, the latter for lower white dwarf masses⁸. All of these hypotheses suggest complete disruption of the white dwarf, with no remnant left and ejection of all of its material, with variable compositions. Also, these models have central temperatures of a few hundred million kelvins and ignite at a central density of about 3×10^9 g cm⁻³.

Our model⁵⁻¹¹ involves white dwarfs which have cooled for

a long time ($t \ge 9 \times 10^8$ yr), so reaching low temperatures ($T \le$ 5×10^7 K) and high central densities ($\rho_c > 5 \times 10^9$ g cm⁻³; this implies a rather high mass: $M \ge 1 M_{\odot}$). Such a situation is likely when the white dwarf's companion star is a low-mass mainsequence star. Then, the detached phase of the system can be very long: $t \ge 10^9$ yr.

The main difference between this and models mentioned earlier is that the carbon-oxygen mixture becomes solid at such temperatures and densities. While carbon ignition in the fluid phase is still an open problem which awaits a two-dimensional

hydrodynamical treatment, solidification introduces important new features.

First, carbon is ignited by pycnonuclear reactions driven by the zero point energy. So, the thermal stability limit is increased to $\rho_0 = 6 \times 10^9$ g cm⁻³. This is still less than the dynamical stability limit, due to electron captures on 16 O, which is ρ_c = 1.92×10¹⁰ g cm⁻³. But an important effect of solidification is to reduce the mechanisms of burning propagation (in the absence of detonation) to conduction alone 12. Because a detonation seems unlikely to form on carbon ignition for densities $\rho \ge 10^7$ g cm⁻³ (ref. 13), solidification thus implies relatively low speeds for burning propagation (~10⁻⁵ times the speed of sound).

To exclude helium detonation in the outer, accreted layers, we limit ourselves to only two accretion regimes: fast accretion

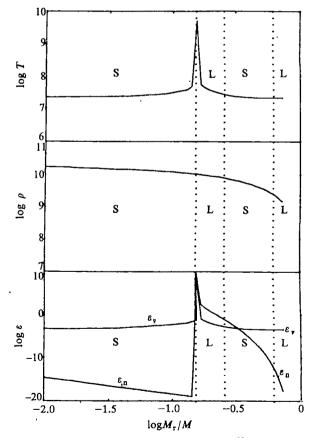


Fig. 1 Stellar structure during the off-centre ¹²C flash. Temperature and density profiles are plotted against stellar mass fraction. Also shown are the energy releases (ε) by the nuclear (fusion) reactions (ε_n) and the e^- captures, together with the thermal neutrino losses (ε_ν) . S and L correspond to the solid and to the melted (Coulomb liquid) zones, respectively.

 $(\dot{M} \ge 10^{-8} M_{\odot} \text{yr}^{-1})$, possibly associated with Roche lobe overflow by the companion, and slow accretion $(\dot{M} \leq 10^{-10} \, M_{\odot} \, \text{yr}^{-1})$, more likely corresponding to accretion from the companion's stellar wind or to decay of the orbit by gravitational radiation.

We have also considered¹² a very fast accretion rate, $\dot{M} \sim$ $10^{-6}\,M_\odot\,\mathrm{yr}^{-1}$ (of the order of the Eddington limit for those stars), and obtained central ¹²C ignition at ρ_c = $1.37 \times 10^{10} \,\mathrm{g \, cm^{-3}}$. Due to the relative slowness of burning propagation in the solid phase, electron captures on the incinerated material overcome the effects of explosive burning and collapse ensues. This study did not include the behaviour of the accreted matter but only its effects in increasing the star's mass and density, and adiabatic gravitational compression. Problems such as the possible formation of an extended red giant envelope¹⁴ or heating of the core by a helium burning shell were not considered. So the real outcome remains unclear and this case serves only to illustrate one of the effects of solidification on pre-supernova evolution. For slower accretion rates those problems may be overcome but ¹²C ignition happens at lower densities $(6 \times 10^9 \text{ g cm}^{-3} \le \rho_o \le 1 \times 10^{10} \text{ g cm}^{-3})$ and electron captures on the incinerated material are also slower. Those cases need further study.

A more important effect of solidification is the possibility of chemical separation. Stevenson¹⁵ has pointed out that the phase diagram of 12C-16O mixtures presents a pronounced eutectic (or temperature minimum in the phase diagram) which corresponds to a composition of 66.8% ¹²C and 33.2% ¹⁶O, both by number. For a 'standard' chemical composition $(X_C = X_O =$ 0.50), the solidification process would undergo the following: (1) 16O 'snowflakes' settling at the centre of the star. (2) The oxygen-depleted fluid is rehomogenized by 'salt finger'-like instabilities. (3) Solid ¹²C begins to form when the fluid composition becomes eutectic. (4) ¹²C crystals, being lighter than the fluid mixture ¹⁶, rise and redissolve higher up. (5) Given enough time, a completely differentiated body is formed.

Note that the cooling process is slowed down by the chemical separation process, as the last involves releasing gravitational potential energy. This effect can be estimated as follows.

The relative change in gravitational potential energy is of the order: $\Delta\Omega/\Omega \sim 10^{-3}$. Typical values for a 1 M_{\odot} star at the beginning of crystallization¹⁷ are: $-\Omega = 4.72 \times 10^{50}$ erg and $L = 10^{-3}$ crystallization¹⁸ are: $-\Omega = 4.72 \times 10^{50}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $L = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 4.72 \times 10^{-3}$ erg and $-\Omega = 10^{-3}$ crystallization¹⁹ are: $-\Omega = 10^{-3}$ cr $1.6 \times 10^{-3} L_{\odot}$. Thus $t = -\Delta \Omega/L = 2.5 \times 10^9$ yr. This means a non-dramatic increase of the time scale of cooling, which was already $t_{cool} \sim 10^9$ yr. It is appreciable, however, and this indicates that partial differentiation when mass accretion begins should be comparatively frequent.

We have calculated the precollapse and/or presupernova evolution of chemically differentiated carbon-oxygen white dwarfs. Our results show that for complete carbon-oxygen separation and for both slow and fast accretion rates (with the reserves previously pointed out concerning very fast rates), the behaviour of the central layers is: (1) heating of the star's centre by electron captures on 16 O, from $\rho_c = 1.92 \times 10^{10}$ g cm⁻³. (2) ¹⁶O melting and building up of a superadiabatic temperature gradient, with convection being stopped by the μ_e -gradient. (3) Growth of 16 C abundance through 16 O +2 $e^- \rightarrow ^{16}$ C +2 ν_e . (4) ¹⁶C ignition when its abundance reaches ~10% by number. The overpressures in the star's centre are small and cannot start a detonation. Burning propagates outwards by conduction alone (the surrounding layers are still solid). The electron captures on the incinerated material being very fast at those densities, ¹⁶C ignition acts as the triggering mechanism for the collapse. Off-centre ignition of ¹²C is still possible during the hydrodynamic compression of the outer half of the star's mass, but this phase is beyond the scope of the present study.

We have also evolved partially differentiated models, in particular those with a central oxygen core surrounded by an eutectic carbon-oxygen mixture ($\sim 0.25 M_{\odot}$ of ¹⁶O surrounded by $\sim 1.15 M_{\odot}$ of ¹²C-¹⁶O). For fast accretion the behaviour of these models is similar to that of the completely differentiated ones. For slow accretion $(\dot{M} = 10^{-12} M_{\odot} \text{yr}^{-1})$, however, ¹²C

ignition happens just at the base of the carbon-oxygen layers. The structure of this model at the time of carbon flash is shown in Fig. 1. Deflagrative carbon burning may, in this case, lead to the ejection of the carbon-oxygen layers after their incineration. The size of the central oxygen core being variable for different degrees of carbon-oxygen separation (corresponding to different cooling times), our model might account for thermonuclear burning of C-O, incineration and ejection of different amounts of material, in the approximative range of $0.5-1 M_{\odot}$. This would agree with Arnett's explanation of the 'fast' to 'slow' SN I range, which involves ejecting from 0.5 M_☉ of ⁵⁶Ni (in the 'slow' SN I) to 1 M_{\odot} of ⁵⁶Ni (in the 'fast' SN I).

Our model, besides leading to neutron star formation, seems also to be a promising explanation for Type I supernovae. The dynamical phase, of mass ejection and/or collapse of the inner zones, must be further explored to ascertain the exact amounts and the chemical composition of the matter expelled. Were our preliminary results confirmed, a single parameter (the duration of the cooling phase) would explain, within the slow accretion regime, the entire range of 'fast' to 'slow' SN I.

Received 23 November 1981: accepted 2 February 1982

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A Compton-cooled feedback mechanism for Cygnus X-1

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The spectrum of the X-ray source Cygnus X-1 appears to exhibit two states. The source spends most of its time in the so-called 'low state' in which it is characterized by a power-law spectrum, $N(\varepsilon) \propto \varepsilon^{-\alpha}$, of photon index 1.6 and high-energy cutoff ~ 100 keV (ref. 1). Occasionally it makes a transition to a high state² during which the soft flux (1-3 keV) increases by a factor of a few and the X-ray spectrum changes to an index steeper than 2.5. Most of the interpretation of the spectrum of Cyg X-1 has centred on the comptonization of soft photons by a hot gas in an accretion disk surrounding a black hole^{1,3}. It has been assumed that the hot gas is held at a fixed temperature, despite the obvious high degree of variability observed in the source on time scales ranging from milliseconds to months^{4,5}. We consider here the effect of relaxing this assumption of constant temperature because a Compton-cooled gas, in which the gas temperature drops in response to the comptonization of soft photons, can readily produce a two-state time-averaged spectrum from variations in the soft flux⁶. We speculate on a feedback mechanism that may effectively lock the source in either of the two states.

The slope and high-energy cutoff of the spectrum in the low state indicate, from computations of Compton-cooling gas⁶ that the Thomson depth τ of the gas is ≥ 2 and that the initial

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gas temperature kT is ~100 keV. Page et al.⁷ measured a lag time (in the high state) for the variations in the 3-40 keV flux compared with the 1-3 keV flux of ~7.5 ms. This is characteristic of the shortest time scales seen in the source apart from the ill-defined submillisecond bursts, the significance of which may be in doubt⁵. Using this lag time scale as a rough guide to the photon escape time, t_e , from the hot gas we find

$$t_{\rm e} \simeq \frac{\tau^2}{n_{\rm e}\sigma_{\rm T}c} = \frac{\tau R_{\rm x}}{c} = 7.5 \times 10^{-3} f$$
 s

where n_o is the electron density, R_x is a typical dimension of a hot gas cloud in the source and f is a factor allowing for the uncertainty in cloud geometry. Thus

$$R_{x} = 1.1 \times 10^{8} \left(\frac{2}{\tau}\right) f \quad \text{cm} \tag{1}$$

and

$$n_e = 2.7 \times 10^{16} \left(\frac{\tau}{2}\right)^2 f^{-1} \text{ cm}^{-3}$$
 (2)

Conservation of energy then implies that the maximum X-ray luminosity

$$L_{\text{max}} \simeq n_{\bullet} (3kT) \frac{4\pi}{3} R_{x}^{3} n_{c} t_{e}^{-1}$$

$$= 10^{37} \left(\frac{2}{\tau}\right) n_{c} f \quad \text{erg s}^{-1}$$
(3)

where n_o is the mean number of clouds present at a given time. The actual luminosity is $(t_o/t_{\rm cool})L_{\rm max}$. (The maximum bremsstrahlung luminosity from this region is almost 100 times smaller.) Observations of L in the low state indicate that fn_o is at least a few^{1,2}. The average scattered photon energy is given by

$$\frac{\langle \varepsilon_{x} \rangle}{\varepsilon_{0}} = \frac{\alpha - 1}{2 - \alpha} \left[\left(\frac{kT}{\varepsilon_{0}} \right)^{2 - \alpha} - 1 \right] \left[1 - \left(\frac{\varepsilon_{0}}{kT} \right)^{\alpha - 1} \right]^{-1} \tag{4}$$

which in the low state, where $\alpha \sim 1.6$ and $\varepsilon_0 \sim 0.5$ keV (refs 2, 8), gives $\langle \varepsilon_x \rangle / \varepsilon_0 = 12$. Then because the observed soft and hard luminosities are similar², we see that only $\sim 10\%$ of the soft photons can be scattered in the hot clouds. We have already noted that τ in the clouds >2, so we conclude that most of the soft photons originate in a region physically separate from the hot gas clouds, ruling out any simple coronal model. The cooling time of a cloud⁶

$$t_{\rm cool} \simeq \frac{3\varepsilon_0}{L_{\rm s}} \exp\left(t_{\rm therm}/t_{\rm e}\right) \frac{4\pi}{3} R_{\rm x}^3 n_{\rm e},\tag{5}$$

where the time taken for photons to scatter to $\varepsilon \sim kT$

$$t_{\text{therm}} = \frac{\log\left(\frac{kT}{\varepsilon_0}\right)}{\log\left(1 + \frac{4}{x}\frac{K_3(x)}{K_2(x)}\right)} (n_{\bullet}\sigma_{\text{T}}c)^{-1}$$
 (6)

 $(x = m_{\rm e}c^2/kT, K_3(x))$ and $K_2(x)$ are modified Bessel functions and L_s is the incident soft photon luminosity at ε_0). Now $L_s \sim 10^{36} n_{\rm e}^{-1}$ erg s⁻¹ in the low state, so for $\tau = 2$ we find

$$t_{\rm cool}^1 \simeq 2 \times 10^{-3} n_c f^2$$
 s (7)

The hard spectrum (together with equation (3) which indicates the value of $n_0 f$) implies that $t_{\infty ol}^1 > t_0$ in the low state. Large variations of the X-ray flux observed on time scales of 20–30 ms (ref. 5) indicate that $n_0 f$ cannot much exceed 10, because a large average number of hot clouds would smooth out variations on time scales of a few times t_0 . In the low state therefore, $t_0 < t_{\infty ol}^1 < 3t_0$ as $\tau \ge 2$. The cooling time is inversely proportional to L_1 , which increases in the high state, so that then $t_0 > t_{\infty ool}^1$. This means that the gas cools significantly before many photons are able to scatter far up in energy resulting in a steeper

spectrum⁶. Comptonized bremsstrahlung, from the rapidly cooling hot clouds, may produce a detectable hard tail to the spectrum in this state. Model spectra have been produced using the method given in ref. 9 assuming an initial gas temperature of 100 keV and predict greater X-ray flux above 100 keV than do the constant temperature comptonization spectra from gas at $kT \sim 30$ keV (refs 1, 10). We can obtain good agreement (by eye) over the 3-500 keV range of the HEAO 1 spectrum from a Compton-cooled model.

The time-averaged spectral index of Compton-cooled gas in the low state is independent⁶ of L_{\bullet} provided that $t_{\infty ol} > t_{en}$ although $L_{\star} \propto L_{\bullet}$. L_{\star} can, moreover, change independently of L_{\bullet} if τ , T or n_{c} change. The spectral index is sensitive to the soft flux in the high state, steepening (flattening) as the flux increases (decreases). The Compton-cooling model can be tested by observing the spectral evolution of a luminous burst. If the temperature of the hot gas is fixed, then the X-ray spectrum of the burst will continually harden with time until it assumes its final shape. If the gas cools, on the other hand, the spectrum will only harden until $t \sim t_{\infty ol}$ (or t_{\bullet} , which ever is the larger) and then it will begin to soften again. The minimum time scale for variations of the soft flux is expected to be the dynamical time scale (of the disk) in the region of its emission³

$$P \simeq 0.5 \left(\frac{M_{\star}}{M_{\odot}}\right)^{-1/2} \left(\frac{R}{10^8 \text{ cm}}\right)^{3/2} \text{ s}$$

We envisage a simplified picture of Cyg X-1 in which soft photons cool clouds of hot gas from $kT \sim 100$ keV on time scales of ~10 ms. The published spectra are the time averages from many clouds which may have passed through many heating (by shocks perhaps) and Compton-cooling phases. The system may lock itself in the two states through feedback provided by the X-ray spectrum. We suppose that the soft photon source is cool gas near to the hard X ray-emitting clouds. The soft photon flux determines t_{cool} and thus the slope of the spectrum of the X rays incident on the cool gas. When $\alpha > 2$ (the low state) the highest-energy photons (~100 keV) dominate this effect which is predominantly heating. This may reduce the soft flux and maintain $t_{cool} > t_o$ and thus $\alpha > 2$. When $\alpha > 2$ (the high state) the lowest-energy photons (>1 keV) dominate and may even have a cooling effect on the soft photon-emitting gas. This may provoke the production of soft photons and maintain $t_{\rm cool} > t_{\rm c}$ and thus $\alpha > 2$. This situation therefore leads to the source latching into either state according to the initial value

To be more specific, we envisage this process occurring in an accretion disk where the X ray-emitting clouds are associated with a thickening due to the local heating exceeding the local cooling rate (the 'thermal instability'3,11). The X rays add a term to either the local heating or cooling rate depending on the spectral slope, thereby increasing or decreasing the size of the X ray-emitting region at the expense of the cool one. The importance of the X-radiation can be estimated by considering a boundary region at R_u of incremental radius h equal to the local half thickness of the cool disk. The flux of X-radiation incident on the boundary is $L_x h/R_u$, which is comparable with the local rate of dissipation of gravitational energy $GMMh/R_u^2$. This last quantity is $L_{\bullet}^{T}h/R_{u}$ or $L_{x}h/R_{u}$, depending on whether the cool region is at a larger or smaller radius than the X ray-emitting region. L_s^T is the total soft X-ray luminosity ($\sim L_x$ in the low state). An increase in M, and thus of L_{\bullet} , of sufficient magnitude to change t_{cool}/t_e , could change R_n and thereby force the system to change its state and remain there until an opposite change occurs. The inferred large value of L_{\bullet}^{T} in Cyg X-1 $(L_s^T > L_x)$ suggests, for an accretion model, that the luminous soft flux originates from an area at a smaller radius than the X-ray region (which requires some special geometry in order that only 10% of the photons are intercepted by the hot gassee Fig. 2 in ref. 12 which illustrates this general configuration; the details are different). This would mean that soft (<2 keV) X-ray measurements of Cyg X-1 are the most direct probes of

the black hole, and that the soft X rays probably control the shortest time-scale variability. Possible alternative solutions are that \dot{M} decreases with radius due to a wind, or that the efficiency is low in the hot region because the black hole swallows much of the kinetic energy with the matter. Clearly our model requires a significant perturbation to drive it from one state to the other. The size of the necessary perturbation required to drive the system from low to high (high to low) will depend on $t_{\rm cool}^{\rm h}/t_{\rm e}(t_{\rm cool}^{\rm l}/t_{\rm e})$. The longer time spent in the low state may simply reflect the greater stability of that state to changes in the soft flux.

We conclude that, in the framework of a Compton-cooled plasma, the flat hard X-ray spectrum and rapid variability observed in the low state of Cyg X-1 imply that the Comptoncooling time, t_{cool} , just exceeds the photon escape time from the hot clouds, t_c . Feedback through the following loop then maintains that low state: $\alpha < 2 \Rightarrow$ emission from hot gas heats surrounding cool gas $\Rightarrow L_s$ decreases $\Rightarrow t_{cool}$ increases $\Rightarrow t_{cool} >$ $t_e \Rightarrow \alpha < 2$. A substantial increase in L_s , perhaps due to a temporary change in \dot{M} , may then cause a change to the high-state loop: $\alpha > 2 \Rightarrow$ emission from hot gas cools cool gas $\Rightarrow L_s$ increases $\Rightarrow t_{cool} < t_e \Rightarrow \alpha > 2$.

A.C.F. thanks the Radcliffe Trust for support and P.W.G. the Guernsey States Education Council.

Received 29 September 1981; accepted 27 January 1982.

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Alkylation of lead (II) salts to tetraalkyllead in aqueous solution

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It was recently reported by Ahmad et al.1 that lead (II) salts react with alkyl iodides in aqueous solution to produce tetraalkyllead compounds. They stated this reaction would have serious environmental consequences for water quality and for the well-being of aquatic biota. The experiments were carried out in a small (250 ml) flask nearly filled with an aqueous lead (II) acetate solution (210 ml) and sealed with an aluminium foil wrapped stopper. We have now repeated the experiment and report that tetramethyllead (TML) is not formed by direct reaction of lead (II) salts with methyl iodide in an aqueous system, but comes from a secondary reaction of metallic lead with methyl iodide2.3. Apparently, Ahmad et al.1 inadvertently contacted the aluminium foil-wrapped stopper with a small quantity of lead (II) acetate solution to produce finely divided metallic lead which reacted with methyl iodide to produce TML. The reduction of lead (II) salts to metallic lead in both acid and alkaline solutions by aluminium and other metals has been reported previously⁴. When the reactions described by Ahmad et al.1 were carried out in the absence and presence of aluminium, TML formed in those tests in which lead (II) acetate solution contacted aluminium but not when aluminium was absent. Our results indicate that methylation of lead (II) acetate does not occur in the environment to form tetraalkyllead compounds which would be deleterious to water quality.

We have repeated the alkylation experiments described by Ahmad et al. by reacting lead (II) acetate with methyl iodide in aqueous solutions adjusted to pH 5-6 and pH 13 respectively. The experiments carried out in alkaline solution at pH 13 are reported in Table 1. Alkylation runs 1 and 2 were carried out as described by Ahmad et al. by dissolving 1.0 mmol of lead (II) acetate in 200 ml of deionized water in a 250 ml filtration flask fitted with an aluminium foil-wrapped stopper. Methyl iodide (0.1 ml) was added and the flask was sealed and stored in a closed cabinet. After 5 days the mixture (vapour space and aqueous solution) was extracted with n-heptane. The TML content of n-heptane was measured by a colorimetric dithizone method⁵ modified to measure the absorbance of dialkyllead dithizonate in a small volume (0.9 ml) of chloroformdithizone solution at 485 nm wavelength. Results from the first two experiments (Table 1) using an independent method of analysis agreed well with those reported by Ahmad et al. 1. In the next three runs, contact between the aluminium foil-wrapped stopper and the lead (II) acetate solution was controlled. The foil in runs 3 and 4 was deliberately and completely wetted with lead (II) acetate solution (pH 13) at the start and occasionally during the experiment. Special precautions were taken to avoid contact between the foil-wrapped stopper and lead (II) acetate solution in run 5. The results of these runs demonstrate that TML is produced only if the aluminium foil-wrapped stopper contracts the lead (II) acetate solution. Significantly more TML was formed when the aluminium foil was intentionally wetted than in runs 1 and 2 where there was minimal and unintentional contact between lead (II) acetate solution and aluminium foil.

As further proof that there is no reaction between lead (II) acetate and methyl iodide in aqueous solution (pH 13) to produce TML, we increased the sensitivity of the colorimetric dithizone method by a factor of 10 by scaling up proportionally the quantities of lead (II) acetate, methyl iodide, aqueous solution and glassware used in the alkylation reaction. The reactants and solvent were maintained in the ratios used by Ahmad et al. 1. Repeat experiments were carried out in the absence of aluminium with the bottles sealed with screw caps lined with Teflon gaskets. No detectable quantities of TML (<0.01 p.p.b. as Pb) were produced from these tests (runs 6-10) carried out over 5-50 days.

Methylation tests were then carried out at pH 5-6. Aluminium was added directly to the lead (II) acetate solution in

Table 1 Reaction of lead ((II) acetate with methyl iodide in aqueous solution (pH 13)

Run no.	Reaction period (days)	Experimental conditions	TML found (p.p.b. Pb in water)
1	5	a	1.2
2	6	a	1.5
3	5	b	8.5
4	5	ь	10.2
5	5	c	< 0.1
6	5	d	< 0.01
7	5	d	< 0.01
8	10	d	<
9	18	d	< 0.01
10	50	d	< 0.01

a, Aluminium foil present and methylation test carried out as described by Ahmad et al. 1. b, Aluminium foil deliberately wetted with lead (II) acetate solution (pH 13). c, Aluminium foil present but kept free of lead (II) acetate solution (pH 13). d, Absence of aluminium foil; the methylation experiments were scaled up proportionally to provide increased analytical sensitivity. In all cases, temperature was 23 ± 2 °C. Note that Ahmad et al. found 370.5 ng of TML in a flask containing 200 ml of aqueous solution, which is equivalent to a 1.44 p.p.b. calculated as Pb in water.

Table 2 Reaction of metallic lead with methyl iodide in aqueous solution

Run no.	Reaction period (days)	рН	TML found (p.p.b. Pb in water)
1	7	13	744.0
2	6	13	2,375.0
3	5	Initially 5.5	< 0.01
4	9	Initially 5.5	< 0.01

Sample no. 2 contained 10 g of freshly sliced metallic lead while sample 1 contained 5 g of lead. However, the weight of lead is less important than the surface area of the freshly sliced lead. Samples 3 and 4 contained 8 g of freshly sliced lead. All tests contained 1,140 p.p.b. of methyl iodide in water. Temperature 23 ± 2 °C.

some experiments and omitted in others. The head space in the 250 ml flask was analysed by a gas chromatography/atomic absorption spectrometry procedure essentially as described in ref. 6 and having the same sensitivity as the method of Ahmad et al.¹. In these experiments, TML was detected only when the aluminium was contacted with lead (II) acetate solution.

That metallic lead is produced from the reduction of lead (II) acetate was confirmed by adding aluminium foil to (1) lead (II) acetate dissolved in distilled water (pH 5.5) and (2) lead (II) acetate solution adjusted to pH 13 with NaOH. The metallic lead produced was identified by scanning electron microscopy/energy-dispersive X-ray spectrometry. The reduction at pH 5.5 is much slower than at pH 13, which may explain why Ahmad et al. found significantly smaller quantities of TML in slightly acid solutions.

Although the reaction of metallic lead with methyl iodide has been reported previously^{2,3}, we have demonstrated here that this reaction depends on the extent and character of the lead surface and the specific conditions in which the test is carried out. When we added freshly sliced metallic lead to a NaOH solution (pH 13) containing methyl iodide, TML was readily produced (Table 2). On the other hand, no TML was detected when freshly sliced lead was added to distilled water (pH 5.5) containing methyl iodide and the mixture allowed to stand in the dark and in a completely sedentary condition for 5-9 days (Table 2). In the latter case a rapid growth of insoluble hydrated lead oxides formed on the surface of the metallic lead, presumably inhibiting the alkylation reactions. In the presence of surface waters, metallic lead would be expected to react with other anions, such as sulphides, carbonates and phosphates, to form water-insoluble lead compounds adhering to the surface of the metal that would reduce or eliminate any alkylation of metallic lead with alkyl iodides, even if they were present in very high concentrations. Caution must be exercised against predicting whether metallic lead may be methylated in the environment by extrapolating information from laboratory experiments, especially as metallic lead and methyl iodide are chemically unstable in water.

When Jarvie and Whitmore⁷ repeated the lead (II) salt-methyl iodide reactions described by Ahmad et al.¹, no TML was observed when the reactions were carried out in the presence and in the absence of aluminium foil-wrapped stoppers. These authors attempted to explain their negative results on the basis of possible minor differences in temperature, time, pH, light effects, for example, from the conditions used by Ahmad et al.¹. However, we believe the correct explanation is that none of the aqueous lead acetate solution inadvertently contacted the aluminium foil-wrapped stoppers.

Received 6 August; accepted 18 December 1981.

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Aerosols of high chlorine concentration transported into central and eastern United States

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During 18-21 December 1980 a remarkable change occurred in the composition of the aerosol over a broad region of the central and eastern United States: the weight per cent of chlorine present in small aerosol particles increased by nearly two orders of magnitude. We report here an analysis of the aerosol composition and meteorology during the period of change which suggests that this increase was the result not of a pollution episode, but of a massive intrusion of very clean maritime air from the northern Pacific into a region extending at least from Wisconsin southwards to Kansas and Missouri and eastwards to Ohio.

We obtained elemental composition data for the event from X-ray fluorescence analysis of aerosols collected using virtual impactor (dichotomous) samplers. These samplers separate aerosols into 'fine' and 'coarse' fractions (<2.5 and 2.5-15 µm in aerodynamic diameter, respectively). Particles in the fine aerosol fraction have settling velocities in still air of ≤10⁻² cm s⁻¹; consequently they may remain suspended for days or longer and may be transported long distances by largescale atmospheric motion. The fractions were collected on Teflon membrane filters over 24-h intervals starting at midnight. Locations where aerosol samplers were operating during the period of interest and for which we have aerosol elemental composition data are three rural sites (in Kentucky, Indiana and Ohio); Portage, Wisconsin; Topeka, Kansas; and St Louis, Missouri¹. The rural sites were chosen as being representative of regional air quality and to avoid local emission sources as far as possible; these sites were located at 37°41' N, 88°01' W; 39°28' N, 84°56' W; and 40°36' N, 82°19' W. Aerosols were collected on odd-numbered days in Portage and Topeka and daily elsewhere. We estimate the analytical precision for Cl aerosol concentrations based on the standard deviation of many blank samples to be 2 ng m⁻³

The chlorine concentrations are shown as a percentage of fine aerosol mass in Fig. 1. The ratios of chlorine mass on the peak day to average chlorine mass for several days before and after the event are: Portage (76), Topeka (48), St Louis (28), rural Kentucky (43), rural Indiana (45) and rural Ohio (15). The peak chlorine concentrations in the fine aerosol fraction exceeded 1 µg m⁻³ at all locations except the easternmost site in Ohio. These are the highest fine aerosol chlorine concentrations that we have found among thousands of samples collected in urban and rural areas across the United States during the past 4 yr.

Two previously identified sources of elevated aerosol chlorine are road salting and vegetative burning^{2,3}, although these seem unlikely explanations for an event covering such a large area, they can be ruled out for other reasons, including aerosol properties. Elevated chlorine levels have been observed in aerosols after salting of roads to remove snow. After road salting, most of the chlorine is found in the coarse fraction; during the event reported here, however, the fine to coarse aerosol chlorine ratio was >2:1. City records show that roads

were salted in Portage on 2, 5, 23 and 29 December; in Topeka during 8-11 December and in St Louis on 23 and 24 December. These dates do not correspond to the observed event.

Vegetative burning causes marked increases in fine aerosol potassium. During the chlorine event, however, the potassium levels did not increase significantly in rural areas and decreased in the most polluted area, St Louis. Burning causes large increases in aerosol carbon; soot is an effective light absorber and renders the aerosol black. During the chlorine event, however, the blackness of the collected aerosol, as measured by light transmission of samples from the rural Indiana site, decreased by nearly an order of magnitude. Finally, when the chlorine aerosol levels increased, the total fine aerosol mass decreased by a factor of 2-3 in those areas where the fine aerosol is usually high due to industrial, residential and vehicle emissions (that is the sites at St Louis and rural Kentucky, Indiana and Ohio); this indicates ventilation by clean air. Ionexchange chromatography showed that the concentration of sodium in the samples from the rural Kentucky site began increasing on 19 December, reached a peak value on 20 December which was 10 times higher than the 17 and 18 December levels, and diminished on 21 and 22 December. The mass ratio of Cl⁻/Na⁺ on 20 December was 1.5, equal to the stoichiometric mass ratio for NaCl. These aerosol compositions are consistent with a large, very clean air mass having moved suddenly into the eastern United States, carrying fine aerosol sea salt.

The meteorological conditions associated with this chlorine event are best described as a typical wintertime cold outbreak. A large, cold, dry anticyclone moved over the area of interest and, by 07.00 h on 20 December, covered the United States east of the Rocky Mountains (all times here are Eastern Stan-

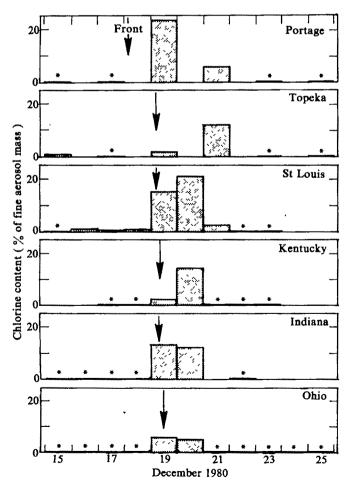


Fig. 1 Chlorine as percentage of fine-fraction aerosol mass collected during 24-h intervals. Note that samples were collected on odd-numbered days at Topeka and Portage. Asterisk indicates <0.5%.

dard Time, EST). The large-scale sinking motion (subsidence) accompanying the cold outbreak brought mid- and upper tropospheric air to lower levels as indicated by an isentropic trajectory analysis⁴. Such a trajectory, plotted backwards in time from 07.00 h on 19 December from a point over Salem. Illinois, showed that air which was at 850 mbar over Salem, was at 548 mbar over Whitehorse in the southern Yukon 48 h earlier. A second, simultaneous trajectory from Green Bay, Wisconsin did not show so much sinking but remained near 700 mbar and 48 h earlier was over Hudson Bay. Note that the trajectory at Salem is for air arriving only a few hours after the frontal passage, while that at Green Bay is for air arriving more than a day after the frontal passage. The stronger subsidence occurring at Salem is consistent with the view⁵ that the strongest subsidence in a cold outbreak occurs shortly after the frontal passage. Thus, the air arriving over our aerosol samplers at 07.00 h on 19 December came from mid-tropospheric altitudes and was over western and central Canada 48 h before. This air was mixed down to the surface by turbulence in the planetary boundary layer. The surface winds in the area of interest were between 5 and 10 m s⁻¹ on the day in question.

The chlorine concentrations reported here are less than those which have been observed in the marine boundary layer, but much larger than those observed in either the continental boundary layer or free troposphere^{6,7}. Consequently we infer that the aerosols are of marine origin. We have not ascertained the exact source of this air, but we speculate that the air was in contact with the open sea long enough to acquire marine characteristics, and was then transported by synoptic-scale motion to the observing sites. There is evidence that air does indeed move from surface to mid-tropospheric levels in frontal cyclones⁵, and we suggest that this rising motion, followed by motion described by the calculated trajectories, was responsible for our observations. Transport of clean air having maritime characteristics to a continental site is the reverse of the transport of continental crustal aerosol to remote marine sites⁸.

Although these observations of high, fine-particle chlorine concentrations are unique as far as we know, nothing in the meteorological conditions seems to distinguish this cold outbreak from others typical of the season. The explanation may be associated with the interesting behaviour of the sulphate component of the fine aerosols which we observed at the Indiana sampling site. Studies in urban areas and in rural areas of the eastern United States have shown that sulphate is generally the most abundant component in the fine aerosol and is largely in the form of ammonium acid sulphate or ammonium sulphate⁹⁻¹¹. Included at the Indiana site was an aerosol sulphate monitor which thermally separated and measured the relatively volatile and non-volatile sulphates in the fine aerosol at intervals of 30 min¹². The non-volatile sulphates are thought to be alkali metal and alkaline earth sulphates, whereas the volatile sulphates consist of ammonium sulphate and ammonium acid sulphate. Preceding the event at the Indiana site, the volatile to non-volatile sulphate ratio was ~2:1. Between 23.00 and 24.00 h on 18 December, the amount of volatile sulphate fell abruptly and the non-volatile sulphate decreased slightly, resulting in a volatile to non-volatile sulphate ratio of 1:4. On the night of 21 December, at the end of the chlorine event, the volatile sulphate began to rise to pre-event levels. It may be that, in most cases, chlorine is lost from marine aerosols transported over long distances inland by reactions with acid gases (nitric acid) and aerosols (sulphuric acid and ammonium acid sulphate). The reaction would release chlorine from the marine aerosol in the form of the volatile gas hydrogen chloride. In our observations the abrupt decrease in volatile sulphate before the chlorine event suggests that there was no reactive loss of chlorine from the incoming marine aerosols.

Thus we have observed a brief event of very high concentrations of fine-mode aerosol chlorine at six locations in the central and eastern United States. Presumably, this was the result of transport from the northern Pacific of marine aerosols in an air parcel which displaced aerosols characteristic of the region.

We thank Philip Haagenson of the National Center for Atmospheric Research for the trajectory calculations, John Spengler and his colleagues at Harvard for supplying some of the aerosol samples, and James Huntzicker and colleagues of Oregon Graduate Center for providing sulphate data. Samples in rural Kentucky, Indiana and Ohio were collected by Mead Technology for the US Environmental Protection Agency. Samples from Portage, Topeka, St Louis were collected by the School of Public Health, Harvard University. F.S.B. was on assignment from the National Oceanic and Atmospheric Administration.

Received 25 November 1981; accepted 20 January 1982.

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Thermoluminescence reveals weathering stages in basaltic rocks

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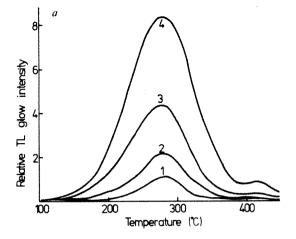
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The weathering of basaltic rocks is important in several regions throughout the world because of its soil forming capacity^{1,2}. Basaltic minerals alter to various types of clay minerals depending on the climatic and chemical conditions of the environment. Investigation of the weathering of rocks involves detailed work consisting of thin section and X-ray and electron microscopy techniques: alternative methods would be desirable. We show here that the thermoluminescence (TL) properties of weathered feldspars can be used as tracers of the relative weathering stages of basaltic rocks.

TL techniques have been used successfully to date pottery³⁻⁵. burned flints⁶, lava flows⁷, young ocean sediments⁸ and biological materials like bone and teeth9. Basically the technique involves the emission of visible light from heated crystalline material, as the electrons in traps are released and recombine with the holes. In minerals and rocks, the ionizing radiation, primarily from the naturally occurring radioisotopes of ²³⁸U, ²³²Th and ⁴⁰K, pumps the electrons into the traps. The TL efficiency and the radiation dose at which light emission of the material will saturate are a complex function of the ion vacancies, interstitial ions, impurities and dislocations that lead to the formation of traps with semicontinuous energy levels.

Using TL for measurement of geological age, however, has led to difficulties because of the complexity of the geological history involving structural stresses, recrystallization and nonthermal fading of high temperature peaks known as the anomalous fading 10

We initially tried to date samples collected from the parent materials of four sites in south-east Turkey, between Urfa and Siverek. However, their markedly different TL characteristics suggested that we should rather investigate their weathering properties. This region is composed of basaltic rocks of Tertiary age covering the northern part of Syria—the Syrian Shield. The



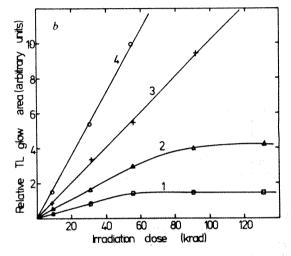


Fig. 1 a, Induced TL glow curves of weathered feldspar from four different profiles of the Karaca Mountain basaltic plateau. The irradiation dose was 55 krad and the heating rate 2 °Cs Low-temperature peaks were erased by preheating the samples at 80 °C for 5 s. b, Variation of TL glow curve area under the 275 °C peak as a function of the irradiation dose. The saturation doses were 55, 80, 130 and 300 krad respectively from layers 1 to 4. Points corresponding to high radiation doses of 3 and 4 are not shown. The numbers on the curves indicate their relative weathering in increasing order.

samples came from profiles typical of topographically and chronologically sequential continuations of different lava flow levels of the Karaca Mountain (1,919 m).

Feldspar grains, which are responsible for producing TL in basaltic rocks, were separated out from the samples. The basalts were viced in a V-shaped metal to extract feldspars without damaging them. They were then washed with HCl to dissolve the secondary CaCO₃ depositions in the vesicles. After dispersing the samples in Calgon overnight, grains of 100-150 µm size were separated by wet sieving. Lighter feldspar grains were extracted by floating them in bromoform. Aliquots of 5-mg feldspar grains were spread over 1-cm diameter aluminium disks for TL measurements. These samples were heated in an argon atmosphere from 100 to 480 °C at a rate of 2 °C s⁻¹ and natural TL glow curves were obtained. It was observed that during this process, the natural TL of all the samples was almost completely drained. Subsequently, varying radiation doses were given to the samples using a 200 mCi ⁹⁰Sr/⁹⁰Yr β source and glow curves were obtained 5 min later. Figure 1a shows the induced TL glow curves of the feldspar samples from the four different profiles of the Karaca Mountain basaltic plateau. Figure 1b shows the variation of the TL glow area under the 275 °C peak with radiation dose. Each point represents the average of four measurements from each sample. The TL sensitivities of the samples were obtained by plotting the linear

^{*} Dr Göksu Ögelman has been dismissed from her post by the Martial Law Authorities in Turkey (see Nature 295, 638).

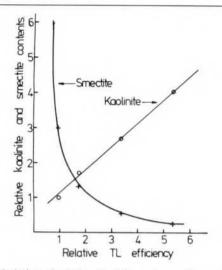


Fig. 2 Variation of relative kaolinite and smectite content with TL efficiency.

portion of the graphs of radiation dose against the area under the 275 °C peak; the saturation dose levels were determined from the asymptote of the curves.

The products from weathering of the same samples of feldspars were observed by polarizing microscope and X-ray diffraction techniques. The relative kaolinite and smectite contents were determined by measuring the area under the 7.2 Å and 18.0 Å peaks respectively, of powdered X-ray diffractograms.

Figure 2 summarizes the relative behaviour of kaolinite, smectite contents of weathered feldspars with respect to TL efficiency. As weathering progresses the kaolinite content increases, the smectite content decreases, and the TL efficiency and saturation dose level increases.

Figure 3 shows a thin section of the basalt from profile 2, covered with feldspars. Weathering can be observed as the cracking of the twinning planes as well as the formation of weathered regions (clay minerals) on the surface. Therefore, it is unlikely that improvement of optical transmission properties could account for the increased TL efficiency. Because the TL efficiency is proportional to the density of lattice defects, it is likely that weathering increases the defects on the surface of feldspars. Scanning and transmission electron microscopy could help to estimate the type of defects produced during the transformation of feldspars to clay with the TL method.

TL is a quick and cheap method compared with the usual lengthy mineralogical procedures for determining the relative

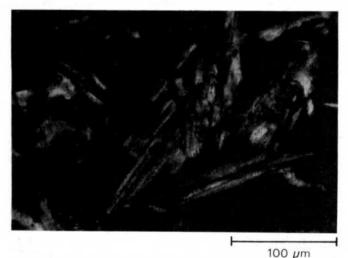


Fig. 3 A thin section of one of the basalt rocks with moderately weathered feldspar grains from profile 2. Weathering can be observed as the cracking of their twinning planes as well as the formation of weathered regions (clay minerals) on the surface.

stages of weathering in basaltic rocks. Conversely, the TL characteristics of weathered basaltic rocks in the same regions or similar climatic and chemical environments, can be used to determine their relative ages. These concepts could be extended to other weathered rock materials.

We thank the Turkish Scientific and Technical Research Council for supporting this research through the Archaeometry Study Group. Y.G.Ö. further thanks her fellow prisoners for friendship and understanding, and her husband whose continued love and support eased the agony of life in prison and made the completion of this article possible.

Received 5 January; accepted 22 January 1982

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A premonitory pattern of earthquakes in northern Greece

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On 20 June 1978 at 20 h 03 min 23 s, an earthquake of surface wave magnitude 6.5 occurred in the Migdonian basin, between the Lagada and Volvi lakes, and 26 km from Thessaloniki. The main shock was preceded by a seismic sequence which started on 8 May. The largest earthquake of that sequence occurred on 23 May and had a surface wave magnitude of 5.8. The long duration of this sequence before the main shock of 20 June had tremendous psychological effects on the whole country. Greek seismologists were in a difficult position during that time (8 May-20 June), especially after the earthquake of 23 May, when pressed to state whether these earthquakes were foreshocks or aftershocks. Long-lasting seismic sequences with earthquakes of magnitude <6 are common in the Aegean area. Because these sequences are sometimes followed by large earthquakes it is important to see whether the latter can be predicted. The May-June 1978 seismic sequence provided the opportunity for testing hypotheses about statistical properties which, it has been suggested, could distinguish between foreshocks and aftershocks. This particular sequence has been investigated extensively 1-4 and more accurate data are available than for any other sequence around Greece. Focal mechanism studies5,6 and field observations² showed that the largest earthquakes of the sequence were produced by horizontal extension in a nearly north-south direction and that the main rupture occurred on a normal fault which strikes almost east-west and dips to the north. The fault length of the main shock of 20 June was estimated7 to be 32 km. Accurate determination of earthquake foci, using portable seismographs, showed that the earthquakes of this sequence were shallow (h < 17 km) while the distribution of the epicentres had a very clear east-west elongation 4.5. A migration of the seismic activity from east to west has also been observed between 8 May and 14 July. We investigate here the time variation of the average magnitude and the frequency of all the shocks with $M \ge 3.0$ of this sequence. The time variations of these seismic parameters are examined in connection with the distribution of well determined epicentres of 38 earthquakes with $M \ge 4.3$ in order to search for any premonitory pattern of the 23 May and 20 June major earthquakes.

Accurate epicentre and focal depth determination for large and small earthquakes of the sequence exist for the period after 3 July 1978 when the first network of portable stations was put in operation in the epicentral region by D. Carver and R. Henrisey of the US Geological Survey. Two other such networks were put in operation, one on 20 July by a team under G. King of Cambridge University in cooperation with the Geophysical Laboratory of the University of Thessaloniki, and another on 1 August by the Public Power Corporation of Greece (PPC). However, one portable seismograph run by the Geophysical Laboratory was in operation in Thessaloniki for the period 26 June-2 July.

For the period 8 May-2 July, the available data come from the network of permanent stations of Greece and neighbouring countries and from a network of three permanent stations of PPC. The closest of these stations are PLG ($40.37\,^{\circ}N$, $23.45\,^{\circ}E$) at a mean distance of $40\,^{\circ}km$ south of the epicentral region, VAY ($41.32\,^{\circ}N$, $22.57\,^{\circ}E$) at a mean distance of $90\,^{\circ}km$ north-west of the epicentral region and the network of the three stations of PPC (KAP: $40.36\,^{\circ}N$, $21.98\,^{\circ}E$, POL: $40.31\,^{\circ}N$, $22.11\,^{\circ}E$, SER: $40.18\,^{\circ}N$, $22.01\,^{\circ}E$) at a mean distance of $\sim 110\,^{\circ}km$ south-west of the epicentral region.

As there was continuous recording from the three PPC stations during the whole sequence, their records were the basis for the present investigation of the time distribution of the earthquakes between 8 May and 2 July. The magnitudes of all these earthquakes were determined using relations of the form M = $a \log \delta t + V \Delta + c$, where, Δ is the epicentral distance⁸ and δt is the signal duration⁸. The constants a, V and c were determined by the usual statistical procedure for earthquakes for which M was known by other means. These formulae were used to determine a magnitude for each station and the average of the three determinations was taken as the magnitude of each earthquake. Magnitudes down to 2.6 were found. In attempting to determine the epicentres for all the earthquakes, we found that for those with $M \ge 2.8$ we could decide whether their epicentres were in the epicentral region of the sequence or elsewhere. However, we finally decided to include in our catalogue and use in this study only earthquakes with $M \ge 3.0$. In this way we have a catalogue of complete and homogeneous data with respect to magnitude for the whole period of the sequence. Between 8 May and 2 July, the number of shocks with $M \ge 3.0$ was 393. This catalogue will be published elsewhere.

The statistical relationship $\log N = a - bM$ between the number, N, of earthquakes which have magnitudes equal to or larger than a certain value and this value, M, holds also for seismic sequences (foreshocks, aftershocks, swarms). The parameter b in the frequency-magnitude relationship is smaller for foreshocks than for aftershocks^{9,10}; laboratory experiments have shown that this can be attributed to the homogeneity of the material¹¹ or to the high stress level¹² in the focal region during the foreshock period. That is why the low values of b have been suggested as precursors of strong earthquakes¹³. However, it is not easy to determine b accurately for several time intervals to follow its pattern, thus the time variation of other parameters, which are related to b but are easily and accurately determined. can be examined to see whether they establish any premonitory patterns of strong earthquakes. The difference in magnitude between the main shock and the largest aftershock has been suggested14 as such a parameter.

We now present evidence that the time variation of the average magnitude value could be considered as premonitory pattern of the 23 May and 20 June 1978 earthquakes. Relationships between \overline{M} and the parameter b have been derived by Hamilton¹⁵ and Utsu¹⁶. Utsu gave the following simple formula:

$$\bar{M} = \frac{\log e}{b} + M_{\min} \tag{1}$$

where $\log e = 0.4343$ and M_{\min} is the magnitude of the smallest earthquake considered. This relation shows that \bar{M} must have relatively large values for foreshocks and smaller values for aftershocks.

The time variation of the average magnitude of this sequence was determined as follows: a minimum number k of events was defined and, for each day, the average of all earthquake magnitudes, with $M \ge M_{\min}$ was taken as the mean magnitude \bar{M} when the number of events n of that day was $\ge k$. When a day had a total number of considered events n < k then the k - n events were taken from its previous day (or days). In this way, each value of \bar{M} was determined with k or more observations.

Such calculations of M were made with all earthquakes in the catalogue $(M_{\min} = 3.0)$ and k = 10. The calculations were repeated with k = 15. Plots of \bar{M} against time have shown that the whole seismic sequence can be separated in four subsequences. These sub-sequences cover the periods 8 May-23 May-1-20 June-2 July, during which the \vec{M} values are 3.67, 3.35, 3.61, 3.48 and the corresponding number of shocks with $M \ge 3.0$ are 24, 57, 65, 247, respectively. This means that the average values of magnitudes are relatively high-low-high-low during these four consecutive time periods. Unfortunately no data exist to determine the average magnitude for the background seismic activity of the area. These data will become available in the next few years because the Geophysical Laboratory installed a telemetry network of eight seismic stations in the area on 1 January 1981. The calculations were repeated with other values of M_{\min} (3.1, 3.2). The decrease in the sample number had some effect on the plots but the basic pattern remained unchanged.

Figure 1a shows the time variation of \bar{M} for k=15 and $M_{\min} = 3.0$. The values for the period 8-13 May have not been plotted because there are not enough data (n < 15) to determine \bar{M} with the accuracy of the other plotted values, but the values

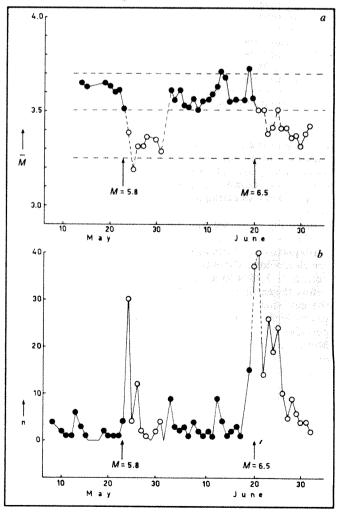


Fig. 1 Time variation of the mean magnitude (a) and of the daily number (b) of the earthquakes of the sequence with $M \ge 3.0$.

• Foreshocks; \bigcirc , aftershocks.

Table 1 Information on all earthquakes with $M \ge 4.3$ of the sequence φN Origin time N Date s.e. Ref. 14:38:58 4040.6 2320.5 40 14 4.7 1 8 May 1978 15:00:08 4044.4 2323.1 10 4.3 14 10 May 1978 13:12:51 4041.5 2322.4 17 4.6 3 2.5 4040.6 13 May 1978 08:35:36 2324.0 11 4.5 23 3 19 May 1978 14:46:08 4041.1 2323.5 15 28 2 23:34:13 4041.9 8 4 23 May 1978 2317.7 5.8 240 1 02:12:30 4039.2 2323.1 14 4.7 84 2 05:57:28 4041.9 13 54 2318.3 46 1 24 May 1978 4043 9 08:13:09 2322.9 4.4 24 4 08:46:31 4042.4 2319.5 4.4 21 3 22:31:25 4042.9 14 4.9 2 2314.6 115 2 June 1978 22:40:13 4044.6 2319.3 6 4.3 25 3 2 2 2 37 17:44:48 4045.4 11 2322.4 4.5 -12 June 1978 23:36:42 4045.9 4.5 2317.2 16 30 13 June 1978 01:36:54 4042.7 2317.6 5 4.3 23 03:12:54 4042.1 2319.6 11 4.5 5.2 21 3 19 June 1978 10:31:07 4042.4 2315.5 12 205 1 10:48:12 4040.9 4.6 87 4 2314 8 11 1 4043.7 20:03:23 2315.2 8 6.5 385 4 20:37:41 4041.8 2309.6 4.4 19 4 20 June 1978 20:45:25 4048.5 2314.7 4.5 22 3 20:52:42 4045.8 17 2309.0 4.3 20 3 2 2 21:51:06 4043.6 2314 9 28 4.6 15 4044.9 03:20:27 2312.4 4.6 23 4039.0 06:00:18 2316.2 4.4 48 3 2 21 June 1978 12:29:46 4044.0 2305.9 0 4.7 94 18:52:06 4041.2 2312.9 10 58 4.4 21:20:14 4043.2 2310.0 4.3 13 20 -4 22 June 1978 4045.7 02:26:42 4 2309.8 4.3 20. 4 2 23 June 1978 01:57:03 4043.0 2304.7 4.5 30 3 26 June 1978 00:04:01 4046.3 2309.1 4.5 27 2 4 July 1978 22:23:28 2 4042.0 2306.4 5.1 148 3 * 6 2 13 July 1978 17:26:57 4040.9 2306.1 4.4 38 4 15 July 1978 4043.8 01:42:03 2318.1 4.3 41 1 21 August 1978 00:52:46 4042.0 2330.1 6 4.3 7 4 4 4.3 24 August 1978 01:23:51 4041.5 2326.7 4 7 2 11 May 1979 01:46:28 4044.0 2321.8 4.7 18 1 31 August 1979 17:24:10 4042.8 2319.8 11 31 2

for this period are clearly high because only shocks with $M \ge 3.2$ occurred during this time. The time of occurrence of the two major earthquakes of 23 May (M = 5.8) and of 20 June (M = 6.5) are also shown in Fig. 1a, while in Fig. 1b the frequency (daily number) of earthquakes with $M \ge 3.0$ as a function of time is shown.

From Fig. 1 we see that during the two aftershock periods (23 May-1 June, 20 June-2 July), when the mean frequency of earthquakes decreases with time, the average magnitude values are clearly low. The mean of the average magnitudes of these periods is 3.38 with a s.d. of 0.06. During both the 15 days before the earthquake of 23 May and during the 19 days before the earthquake of 20 June, the mean magnitude values are clearly high. The mean of the average magnitudes of these two periods is 3.60 with a s.d. of 0.08. It seems, therefore, that the 23 May earthquake was preceded by its foreshocks and followed by its own aftershocks. The latter were distinct from the foreshocks of the 20 June earthquake. The standard deviations above mentioned have been multiplied by 1.6 to determine approximately the 90% confidence intervals. The three horizontal dashed lines in Fig. 1a show these 90% confidence intervals. The upper limit of this interval for the aftershocks coincides with the lower limit of this interval for the foreshock period.

The high values of \overline{M} during the whole seismic period before the earthquake of 23 May as well as during the 19 days period before the earthquake of 20 June could be considered as precursors of these two earthquakes. Similar observations in the future could possibly be used as a warning signal.

The frequency of aftershocks after the main shock of 20 June reached its first minimum on 2 July and therefore the earthquakes which occurred during this period can be considered as

real aftershocks. That is why we considered here only shocks which occurred up to 2 July. The number of shocks with $M \ge 3.0$ for the periods 3-31 July, 1-31 August and 1 September-31 December 1978 was 19, 26 and 31 respectively. That is, the number of shocks are less than one event per day even for July and August. So, it is impossible to determine reliable daily values of \bar{M} and plot them. Instead, the mean magnitudes for each of these periods have been determined and values of 3.49, 3.49 and 3.43 have been found. These values are within the 90% confidence intervals for aftershocks but are relatively high, especially the first two. This is probably due to the expansion of the activity to the west during July and to the east during August in areas which were not previously affected and some earthquakes of these periods considered as aftershocks of the 20 June event were probably foreshocks of second order sequences.

Unfortunately, no network of portable seismographs was operating in the epicentral area before 3 July with which to investigate the space-time distribution of a large number of earthquakes during 8 May-2 June. However, some of the largest earthquakes of this period have been determined accurately^{3,4} using the joint epicentre method. As these determinations are not sufficient to show the space-time distribution of the earthquakes which occurred before 3 July 1978, we have attempted to determine as accurately as possible the epicentres of all earthquakes of the sequence with magnitudes larger than a certain value. For this purpose the Hypo 71 program has been used with a three layer crustal model which is usually applied to determine epicentres in the Aegean area. To increase the accuracy, time delays determined from data of earthquakes recorded by portable seismographs (after 2 July) were added to the arrival times at the permanent stations in Greece and

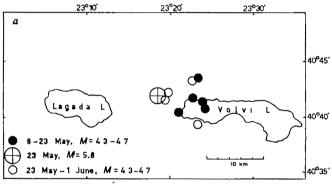
^{*} Our determinations.

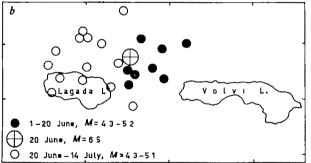
neighbouring countries. It was found that the epicentres of all shocks of the sequence with $M \ge 4.3$ were determined with standard errors ≤4 km. On the other hand, comparison of 20 epicentre determinations made by this method and the determinations of the same epicentres by other methods and data (joint epicentre, portable seismographs) showed very good agreement.

Table 1 gives information for all earthquakes of the sequence with $M \ge 4.3$. The number of observations, N, used to determine the focal coordinates, and the standard error, s.e., of the epicentre are given. All these 38 epicentres are plotted in Fig. 2.

The epicentre of the earthquake of 23 May as well as the epicentres of its largest $(M \ge 4.3)$ foreshocks and aftershocks are shown in Fig. 2a. The epicentres of these earthquakes (8 May-1 June) are located in the eastern part of the seismogenic volume of the entire sequence, near the western coast of the Volvi lake.

Figure 2b shows the epicentre of the main shock of 20 June, the epicentres of the largest $(M \ge 4.3)$ earthquakes which occurred during the 19 days period which preceded this earthquake (1-20 June) and the epicentres of the largest $(M \ge 4.3)$ earthquakes of the period 20 June-14 July. The largest earthquakes of the period 1-20 June had epicentres in a volume close to the epicentre of the main shock north of the area between the two lakes. This supports the idea that these earthquakes are real foreshocks of the earthquake of 20 June. Note that the largest two of these foreshocks occurred near the beginning (2 June, M = 4.9) and the end (19 June, M = 5.2) of this period and were located very close to the epicentre of the main shock. The largest aftershocks of the 20 June earthquake





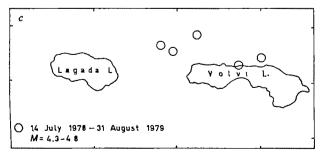


Fig. 2 Epicentres of all earthquakes of the sequence with $M \ge 4.3$ for the periods 8 May-1 June, 1978 (a), 1 June-14 July, 1978 (b) and 14 July, 1978-31 August 1979 (c).

which occurred for 23 days after the main shock were located further west in a volume clearly different from the foreshock volume. Such a westward migration of seismic energy release is also clearly shown in the distribution of the macroseismic intensities.

Figure 2c shows the epicentre distribution for the largest $(M \ge 4.3)$ earthquakes which occurred after 14 July 1978. Note that the main activity during this period shifted to the eastern

The distribution of epicentres shows clearly that between 23 May and 1 June only the eastern part of the seismic volume was active. During this period as well as during the rest of the time until the occurrence of the main shock, the central part of the area, where this main shock of 20 June was located, was in very high stress condition. Because the main seismic activity of the period 1-20 June occurred in this central part of the seismic volume in high stress conditions, high values of \bar{M} (low values of b) are expected during this period. Therefore, the high M values (or low \hat{b}) as well as the space-time distribution of the earthquakes which occurred between 1 and 20 June show that these are real foreshocks. If this information had been known in June before the main event (M = 6.5) it would have helped seismologists to predict that the danger was not then over.

We thank the Public Power Corporation of Greece for use of the records from its stations.

Received 8 October, accepted 31 December 1981

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Direct evidence of a subducted plate under southern Mexico

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The hypothesis1 of subduction of oceanic plates under active continental margins has become a fundamental idea in plate tectonics. Yet the evidence for the existence of oceanic plates beneath continental borders is still largely circumstantial. In seismology, the presence of a new layer or structural unit is taken as confirmed only after seismic phases corresponding to reflections or refractions from that layer have been identified on selsmograms. I report here the presence of a mantle phase, interpreted as a seismic wave refracted from a dipping interface on records of aftershocks from the intermediate earthquake of 24 October 1980 in the Mixtec Highlands of south-central Mexico which may be direct evidence on the structural position of the subducted plate under the Mexican active continental margin.

The destructive earthquake of 24 October 1980 in the Mixtec Highlands ($M_L = 7.4$, h = 70 km) generated over 50 aftershocks

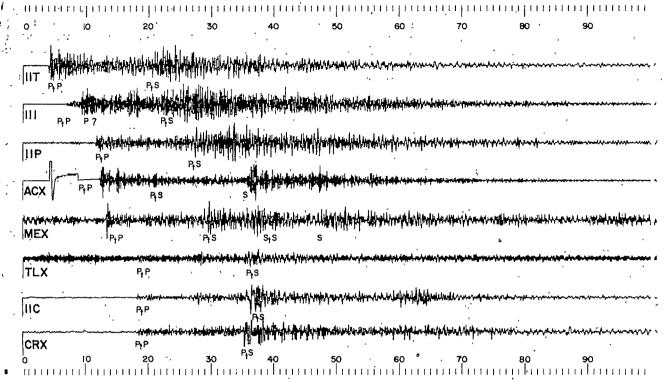


Fig. 1 Seismograms of aftershock 15:076° of the RESMAC catalogue, showing plate phases. All records are from 1-Hz vertical instruments (time in seconds).

which were large enough to be recorded and located on the RESMAC telemetric network. On each record a prominent arrival is found between 10 and 20 s after the initial P signal (Fig. 1). This phase cannot be identified as S, in spite of its low-frequency content, because S is expected to arrive (and is actually seen on most seismograms) 10 or more seconds later. It is not a P-to-S conversion at the bottom of the crust, as this would require a crustal thickness of >100 km and a refraction profile measured in this region in 1975 reveals that the crust is ~40 km thick² (S. K. Singh et al., in preparation).

The possibility that the mantle phase, which we call P_tS, might represent a crustal phase must first be discarded. The focal depth

of 70 km reported by Boulder on the strength of depth phases has been questioned²; however, independent evidence on the focal depth of the aftershocks exists from the records of a four-station temporary network operated in the epicentral region by the Institute of Geophysics of the National University of Mexico (R. Mota, in preparation). The S-P times at the nearest station are typically 7-10 s, indicating (if 40 km is the correct crustal thickness) a range of 55-85 km in focal depths of the aftershocks. If the aftershock activity had been in the crust, S-P times of <6 s should have been observed. The character of the seismograms also indicates a focus below the crust, because of the sharp P-onsets and the absence of the crustal arrivals

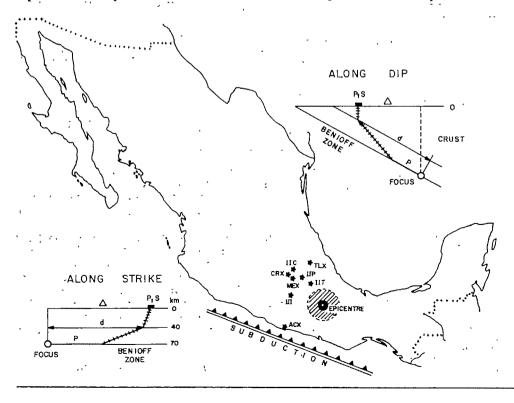


Fig. 2 Location map of RESMAC network in relation to the epicentral region, and proposed path of head wave P_tS refracted from the subducted Cocos Plate. Δ, epicentral distance; d, reduced distance for the same station.

which are typically found on seismograms from shallow events in this region.

The prominent amplitude and low-frequency content of the new phase suggest the presence of a refractor or waveguide at some depth below the crust. If the focus of the earthquake is within or close to the waveguide much of the seismic energy can be trapped and later refracted upwards as P or as S. This waveguide has exactly the structural position and velocity expected for the subducted Cocos Plate. Thus no plausible mechanism except subduction will account for the observations (Fig. 2).

Consider first the conventional travel-time plot constructed for the aftershock (Fig. 1). This event (25 October 1980 at 09:54:38UT) was relocated at 17.98° N, 98.12° W with the aid of the temporary stations. The travel times (Fig. 3) reveal that the apparent velocity of the second arrival is $\sim 8.0 \text{ km s}^{-1}$, too high for any direct or refracted second arrival from a layered crustal model. The expected arrival times for P_g and S_n are shown for comparison. Moreover, the converted P-to-S phase should arrive earlier, that is 6 s after the direct P arrival. Note that the fit of some individual arrivals is poor, notably for the coastal station ACX (Acapulco).

Assuming that the observed second arrivals represent a mantle phase, I present two different models for a deep refractor with a P-velocity of 8.2-8.5 km s⁻¹, corresponding to the bottom of the subducted oceanic crust (Fig. 4). First a simplified model is assumed involving two parallel dipping interfaces. The upper interface is the continental Moho and the lower interface is the oceanic Moho within the subducted plate (Fig. 4a). The geometry is such that the oceanic Moho crops out along the trench axis and the crustal thickness is 40 km at the epicentre. Moreover, the focus of the earthquake is assumed to lie at a depth of 70 km, precisely near the bottom of the subducted oceanic crust. The abscissae are distances measured along the dipping continental Moho, not along the surface of the Earth (see inset, Fig. 4a). In other words, the 'reduced distance' is the intercept of the refracted ray on the continental Moho, used as a datum plane. The Earth's surface cannot be used as datum because the refracted wave fronts describe a set of confocal ellipses on the surface: hence the plotting distance for a given wavefront varies with azimuth, and no rectilinear travel-time plot would be obtained.

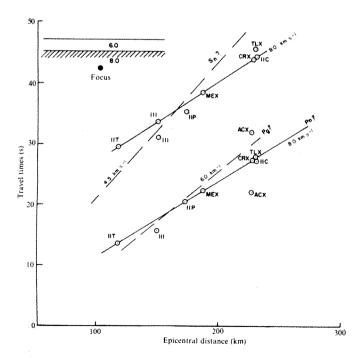


Fig. 3 Travel-time plot for a focus below the crust. Second arrivals do not fit any known phase. Note the poor fit of ACX.

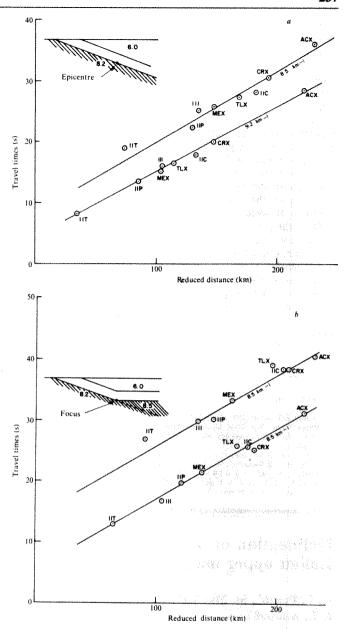


Fig. 4 a, A structural dip of 17° inland from the Mid-America Trench produces a good fit of the data, though the apparent velocities do not check with the model. b, A dipping subduction zone which levels off inland. The second arrival at III is doubtful (see Fig. 1), and might correspond to a direct S. Note that the apparent velocity of second arrivals is independent of the model.

A notable improvement of the travel-time fit is obtained by letting the deep refractor level off inland, as shown in Fig. 4b. This structural position was suggested by recent findings from refraction experiments on the coast of Colombia³. A slightly higher velocity for the inland position of the refractor $(v_p = 8.5 \text{ km s}^{-1})$ was assumed to conform with the Colombian results, though this is not an essential feature of the model. The apparent velocities of both refracted waves $(P_t P$ and $P_t S)$ are now equal and consistent with the model.

Mantle phases from intermediate and deep-focus earth-quakes had previously been observed in Tonga, Japan and Peru⁴⁻⁸, though most of this work describes individual arrivals of phases other than P_tP or P_tS. The RESMAC array in central Mexico is well placed for observations of deep mantle phases; the apparent velocities of the phases can be determined and the approximate structural position of the deep refractor can be inferred. This proof of subduction is particularly convincing to the seismologist as it complements the structural information obtained from offshore explosions recorded on land.

The refractor is tentatively identified as the bottom of the subducted oceanic crust. The oceanic crust itself has a lower velocity, perhaps of the order of 6.8 km s⁻¹. If no such lowvelocity lid existed the refractor would not function efficiently as a waveguide.

Consistently similar seismograms were recorded for over 50 aftershocks of the 1980 Mixtec earthquake. Similar deep refractions have also been observed on other Mexican intermediate-focus seismograms written on the RESMAC array. So-called 'early S-phases' recorded at local stations for the destructive Puebla-Veracruz earthquake of 28 August 1973 (h = 84 km) can probably be interpreted as P.S. The present work demonstrates the potential of mantle phase observations for mapping the structural position of subducted plates under continental borders

Does the subducted Cocos Plate level off inland, as indicated by the model of Fig. 4b? Earthquakes under the coastal plain of Veracruz, east of the Mixtec epicentre of 1980, typically have focal depths in the range of 80-100 km suggesting that the Benioff zone does indeed level off, because substantially greater depths would be expected for a uniformly dipping model as shown in Fig. 4a. Future studies of PrS observations from intermediate shocks in Veracruz should help resolve this question.

The RESMAC seismic network is supported by a grant of the Consejo Nacional de Ciencia y Tecnología of the Government of Mexico.

Received 21 July, accepted 30 October 1981

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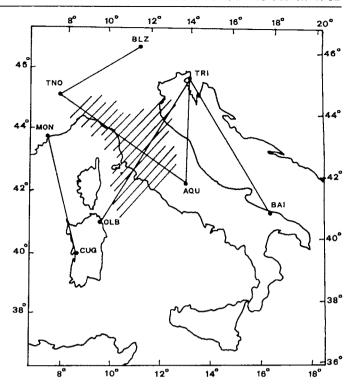
Delineation of the North Central Italian upper mantle anomaly

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The Italian peninsula is the focus of intense deformational tectonic activity. It is underlain by mantle material whose inferred, relatively anomalous properties^{1,2} are not inconsistent with the seismic, volcanic and thermal activity that is manifested in this region. The most effective geophysical tool for mapping the structure of the uppermost 200-300 km of the Earth is the observation and analysis of seismic surface-wave dispersion on a regional scale. Here we synthesize the interpretations of Rayleigh wave dispersion measurements made by several authors, each for different parts of North Central Italy3delineate the lateral extent of the upper mantle anomaly in this

Over the past 20 years intensive efforts have been made to study details of the cross-section of the crust and upper mantle in the European-Mediterranean area. As a result of these studies, several zones were delineated^{1,2}, such as the Western Mediterranean, the Tyrrhenian Sea, the Adriatic promontory of the African plate and the Central European Rift System, each with differing mantle structure. In addition to these struc-



Surface wave paths relevant to this study. The shaded region is the location of the mantle anomaly in North Central Italy.

tures, another conspicuous anomaly can be identified under the north-central part of the Apennine peninsula, that is the tectonically active region to the east and north of Corsica.

The properties of the upper mantle under North Central Italy (Fig. 1) have been delineated by Rayleigh wave dispersion measurements along two profiles TNO-AQU and TRI-OLB^{5,6} These profiles have unusually low phase velocities in the period range 40-60 s (Fig. 2). Velocities as low as these have been observed in the Western USA9, as, for example, on the path TUC-BOZ, and the East African Rift¹⁰, both regions of high heat flow. The correlation between the surface wave phase velocities in northern Italy and high heat flow is even stronger after the phase velocities have been inverted; cross-sections are found consistent with the absence of a lid to the low-velocity channel (Fig. 3) and anomalously low S-wave velocities that extend up to, or close to the Mohorovicic discontinuity. In these cases the high surface heat flows are probably thus derived from high temperatures at shallow depth in the mantle and hence are a surface expression of tectonic involvement on a scale extending into the mantle.

The phase-velocity profile TNO-AQU (Fig. 2) differs in shape from the other two examples and would seem to provide an example of the danger of a literal interpretation of phase velocity curves in the period range 40-60 s. This curve differs from the others because of the influence of a larger crustal thickness under the axis of the Apennines than in the other regions described by low-phase velocities. Inversion of this phase velocity curve indeed indicates that a lid to a low S-wave velocity channel is absent⁵, and that this curve is properly catalogued with TRI-OLB, TUC-BOZ and others.

We can provide a rough limit to the lateral extent of the Italian anomaly. To the south, the region is abutted by the Tyrrhenian Sea and still farther south by the Sicilian crossing of the Mediterranean by the contact between the African and European plates. High heat flows, volcanism, low surface-wave phase velocities, deep focus earthquakes, and an inferred subduction¹¹ give the Tyrrhenian region the appearance of a backarc basin. Thus the anomaly of the northern Italian peninsula seems to continue southward; the southern limit to the Tyrrhenian extension of the anomaly would appear to be at or

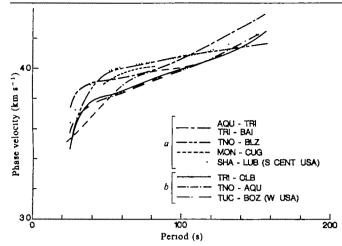


Fig. 2 Phase velocities used to delineate the North Central Italian anomaly and comparison with dispersion relations for western and South-Central USA. The r.m.s. errors for each curve are $\sim \pm 0.03 \text{ km s}^{-1}$ in the period range 40-60 s and are larger at the extremes of the period range $^{3.5-9}$. Curves in group a are for regions without tectonic involvement and in group b for regions with tectonic involvement.

near the plate boundary with North Africa. An exploration of this region will be described elsewhere.

To the west, surface-wave profiles near Corsica and Sardinia on path MON-CUG³, as well as to the north in the Italian Alpine foothills on path TNO-BLZ^{4,7}, have shown rather higher phase velocities in the period range characteristic of mantle depths. Similar phase velocities have been found elsewhere in relatively older, stable continental regions with more-or-less normal heat flows, that is, regions without major tectonic involvement today^{9,12,13} including the US Gulf Coast, such as on path SHA-LUB, and Western Europe. While inversion of these latter phase velocities may differ in some detail, especially in view of the non-uniqueness of the inversions, all successful inversions yield an upper mantle cross-section which has a broad low-velocity channel of significant contrast to an overlying high-velocity lid (Fig. 3); the channel-lid interface is found around a depth of 100 km. As indicated, this cross-section is evidently well correlated with the age and stability of these regions, characterized by their 'normal' heat flows and relatively

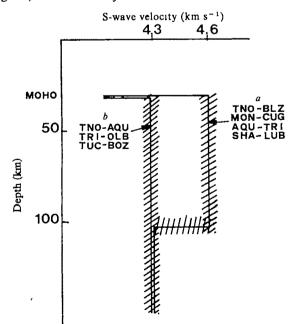


Fig. 3 Schematic shear wave velocity-depth distributions in the upper mantle for regions without a and with b major tectonic involvement today. The shading represents schematically the range of models satisfying each group of dispersion data.

low seismicity.

The values of phase velocities at periods between 40 and 60 s are diagnostic between the two groups. The errors in measurement are in every case less than the phase velocity interval between the groups of curves. It follows that the inversions of these phase velocity data yield structures for the two typical regions that are completely resolved from one another; the differences between the geophysical and geological environments of these two groups of regions are equally large.

The eastern edge of the Italian anomaly is delineated by surface-wave phase velocities that were determined along two Adriatic two-station paths, TRI-BAI and TRI-AQU⁸. The two phase velocity curves are indistinguishable over their common period range and are plotted as a single curve in Fig. 2. They are clearly members of the family of curves associated with young, stable continental regions, such as those for Western Europe. Such regions have well defined lids to channels. Crosssections such as those for northern Italy, in which high-velocity lids are absent, are excluded. The inversion of these curves is no different, within errors of measurement, from those derived for other regions and sketched in Fig. 3.

We conclude that the anomalous low phase velocity region of northern Italy lies to the east of the path MON-CUG, to the west of the path AQU-TRI, and south of the Alps.

This work was supported by grants from CNR, Rome (80.2213) and NATO (1497), the Deutsche Forschungsgemeinschaft and the Schweizerischer Nationalfonds zür Forderung der wissenschaftlichen Forschung.

Received 20 July 1981, accepted 19 January 1982

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Pb isotopic composition of ophiolitic volcanogenic sulphide deposits, Troodos Complex, Cyprus

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The volcanogenic sulphide ore deposits in the ophiolitic rocks of the Troodos Massif, Cyprus, are thought to be ~80 Myr old¹ analogues of the metal sulphide accumulations actively being deposited from ~350 °C water at a depth of ~2.6 km on the East Pacific Rise (21°N)²⁻⁴. In both cases, mineralization is thought to be a consequence of heat transfer by convective seawater circulation within the oceanic crust^{5,6}. Evidence includes fluid inclusions in quartz from ore deposits in Cyprus with modal trapping temperatures of 298-370 °C which have a bulk composition of 3.2 \pm 0.5 equiv. wt % NaCl (1 σ ; n=273). This salinity is identical to seawater with 3.5 wt% total dissolved solids^{4,7}. We have examined the isotopic composition of Pb as a geochemical tracer to clarify the origin of one of the metals contained in the sulphide concentrations in the Troodos Complex. It was anticipated that the bulk of the lead would have been derived by leaching of the mafic ophiolitic rocks and, indeed, such a component is found to be present. However, the isotope ratios suggest that the lead is actually a mixture, and also contains an important component derived from seawater.

Interpretation of the isotopic data for the sulphides requires a knowledge of the isotopic characteristics of the associated igneous rocks of the ophiolitic complex. Hence, Pb isotope ratios were determined for a suite of eight variably ocean floor-metamorphosed basalts, dolerites and gabbros, the precise locations of which are given in Table 1 together with the results. Initial $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ ratios were determined to monitor the degree of seawater-rock interaction which might also have affected the Pb isotopic composition, as this process is associated with increases in the $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ ratios of altered rocks above initial magmatic $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ ratios of 0.70338±0.00010 to 0.70365±0.00005 (ref. 8).

Sulphide Pb isotope ratios were determined for 11 samples from five deposits (Table 2). In addition, a single independently obtained set of values is available for Skouriotissa9. As can be seen in Fig. 1, these six deposits give good spatial coverage of the Troodos Complex. In general terms, the sulphide concentrations consist of lenses of massive ore intercalated conformably. within the pillow lava succession which are underlain by mineralized stockworks⁴. Massive ore is thought to have precipitated on the actual ocean floor from hydrothermal solutions which discharged through the stockworks beneath, and was deliberately avoided in this study because of possible complications associated with high level seawater mixing. Hence, most of the samples (10 out of 12) are from stockworks some of which are deep (for example, Alestos; ~1km), others being relatively shallow (for example, Mathiati; ~40 m) relative to the original ocean floor. Two samples from the Limni mine in western Cyprus were mill concentrates and represent an average of the ore being processed at the time. All the other materials analysed were sulphide separates consisting mainly of pyrite and chalcopyrite produced from ~0.5 kg samples.

As shown in Fig. 2, most of the lead isotope compositions of the ophiolitic mafic rocks fall in, or near, the fields for mid-ocean ridge basalts¹⁰, or are less radiogenic. Some very low Pb isotope ratios were determined. For example, two gabbros (T74 and T137) have 206Pb/204Pb, 207Pb/204Pb and ⁸Pb/²⁰⁴Pb ratios of 16.620 and 17.050, 15.401 and 15.481, and, 36.511 and 36.801, respectively. A metadolerite (CY/75/80) has ratios of 17.398, 15.370 and 36.930. These values are all distinctly less radiogenic than any presented by Tatsumoto¹⁰. For some reason, two of the samples (CY/75/73 and 94B) seem to have slightly higher ²⁰⁷Pb/²⁰⁴Pb ratios than is typical (Fig. 2b). The Pb isotopic compositions of most of the samples are not likely to have been significantly affected by seawater interaction because sample CY/75/80, a metadolerite dyke with a contaminated initial 87Sr/86Sr ratio of 0.70505±0.00003, has unradiogenic Pb isotope ratios similar to fresh gabbros with initial 87 Sr/ 86 Sr ratios of 0.70357 ± 0.00006 and 0.70362 ± 0.00006 , and a metabasalt sample (CY/75/75; no. 1 in Fig. 2) with a low initial 87Sr/86Sr ratio of 0.70342 ± 0.00003 has Pb isotope ratios which are near the radiogenic end of the defined range. In addition, the most Sr-isotopically contaminated sample (CY/75/94B; no. 8 in Fig. 2), with an initial ${}^{87}Sr/{}^{86}Sr$ ratio of 0.70585 ± 0.00003 , has initial Pb isotope ratios which are slightly less radiogenic than sample CY/75/75 (no. 1) discussed above. A possible exception is sample CY/75/71 (no. 7 in Fig. 2) with the most radiogenic Pb isotope ratios which plot between the mid-ocean ridge basalt fields and the seawater fields. This sample was notable because it contained a prominent hydrothermal epidote-sulphide vein which could have contained Pb dissolved in the circulating fluid of seawater origin. The Pb isotopic composition of the ophiolitic mafic rocks of the Troodos Complex is apparently comparable with that of mid-ocean ridge basalts, but also extends to lower

The Pb isotope ratios of the 11 sulphide separates from five ore deposits analysed in this study are more radiogenic than those for the ophiolitic mafic rocks (Table 2 and Fig. 2). The following ranges are defined: $^{206}\text{Pb}/^{204}\text{Pb} = 18.404-18.888;$ $^{207}\text{Pb}/^{204}\text{Pb} = 15.551-15.634;$ $^{208}\text{Pb}/^{204}\text{Pb} = 38.271-38.743.$

Table 1	Sr and Pb isotope ra	tios for mafic ophiol	itic rocks from	n the Troo	dos Comple	x, Cyprus, listed	d in order of i	ncreasing ⁸⁷ Sı	·/ ⁸⁶ Sr ratio
Sample no.	Sample description	Sample locality	Rb (p.p.m.)	Sr (p.p.m.)	Rb/Sr	⁸⁷ Sr/ ⁸⁶ Sr	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb
CY/75/75 (1)	Metabasalt with SiO ₂ amygdales	Aia Marina- Xyliatos	~1.3	88.8	~0.015	0.70342± 0.00003	18.246	15.495	37.941
T74(2)	Fresh gabbro	3 km east of Pedhoulas		_		0.70357±* 0.00006	16.620	15.401	36.511
T137(3)	Fresh gabbro	200 m west of Troodhitissa monastery	***************************************	,		0.70362±* 0.00006	17.050	15.481	36.801
CY/75/73 (4)	Metadolerite dyke	1 km west of Stavros tis Psokas	~0.7	~47	~0.016	0.70470± 0.00004	18.173	15.549	37.809
CY/75/94A (5)	Metagabbro	Agros	~0.3	82.5	~0.003	0.70475 ± 0.00005	18.207	15.480	37.648
CŶ/75/80 (6)	Metadolerite dyke	Xyliatos-Spilia	~0.4	~123	~0.004	0.70505 ± 0.00003	17.398	15.370	36.930
CÝ/75/71 (7)	Metadolerite dyke with epidote-sulphide vein	Lyso-Stavros tis Psokas	~0.5	~481	~0.001	0.70538± 0.00004	18.549	15.609	38.437
CY/75/94B (8)	Metadolerite dyke	Agros	~0.2	152	~0.001	0.70585 ± 0.00003	18.115	15.599	37.802

Rb and Sr were determined by X-ray fluorescence on a Philips 1410 instrument²⁰. Sr was extracted using standard ion exchange techniques and its isotopic ratio was analysed on a V.G. Micromass 30 mass spectrometer²¹. As all samples were found to have Rb/Sr ratios <0.02 age correction to 80 Myr BP, the age of the Troodos Complex¹, was unnecessary. Quoted errors are 2 s.d. and are ≤ 0.00005 . All results are quoted relative to an Eimer and Amend SrCO₃ standard isotopic ratio of 0.70800. The mean Eimer and Amend 87 Sr/ 86 Sr ratio measured in this work was 0.70807 ± 0.00002 . Pb was extracted using anodic electrodeposition²² and its isotopic composition analysed with a 12-inch radius mass spectrometer using a standard silica gel-phosphoric acid technique which yields ratios accurate to better than $\pm 0.1\%$. The values have not been age corrected to 80 Myr BP using independently determined U/Pb and Th/Pb ratios. Hence, initial ratios would be less radiogenic.

* From ref. 8.

Table 2 Pb concentrations and isotope ratios for sulphide separates from volcanogenic ore deposits, Troodos Complex, Cyprus

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Sample no.	Sample locality	Locality description	Pb (p.p.m.)	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb
CY/75/98A	Alestos	Deep stockwork in basal group	1.55	18.404	15.560	38.271
CY/75/98D	Alestos	8	3.3	18.480	15.563	38.367
_	Skouriotissa*	Massive ore	176	18.476	15.571	38.405
CY/75/91	Peristerka (New Kambia)	High level stockwork	1.7	18.526	15.564	38.441
CY/75/12	Limni	Medium level stockwork	12.9	18.525	15.616	38.518
CY/75/33	Limni	Medium level stockwork	26.9	18.543	15.595	38.420
CY/75/69	Limni	Medium level stockwork Pyrite concentrate	39.0	18.546	15.634	38.599
CY/75/70	Limni	Chalcopyrite concentrate	ND	18.524	15.600	38.481
CY/75/87	Mathiati	High level stockwork	15.2	18.661	15.617	38.676
CY/75/89	Mathiati	Massive ore	30.4	18.658	15.599	38.651
CY/75/100B	Mousoulos	High level stockwork	3.6	18.887	15.587	38.743
CY/75/100C	Mousoulos	High level stockwork	5.6	18.888	15.551	38.612

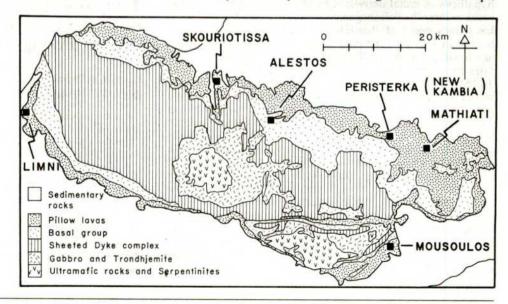
Pb concentrations were determined by mass spectrometric isotope dilution with an accuracy of $\pm 2\%$. Either a solvent extraction step using methylisobutylketone (MIBK) or an HBr anion ion exchange column was used to remove Fe before extraction of lead by anodic electrodeposition²². Isotopic analysis was carried out by the method described in Table 1. Age correction to 80 Myr BP was unnecessary as sulphides contain very low levels of U and Th relative to lead.

* From ref. 9.

Lead in the sulphide separates is present at trace levels of 1.55-39.0 p.p.m. averaging 14.0 ± 13.6 p.p.m. $(1\sigma; n = 10)$. These low concentrations reflect the lead-poor nature of ophiolitic volcanogenic sulphide deposits in contrast to Kuroko volcanogenic deposits which typically contain ~1% Pb, and are actually lower than lead concentrations of 115.0 ± 56.7 p.p.m. $(1\sigma; n=18)$ determined by isotope dilution for Fe-Mn hydroxyoxide metalliferous sediments associated with ophiolitic rocks in Cyprus, Syria and The Sultanate of Oman11. An independently determined set of ratios for the Skouriotissa deposit fall satisfactorily within the above ranges with corresponding values of 18.476, 15.571 and 38.405 (ref. 9). On ²⁰⁸Pb/²⁰⁴Pb against ²⁰⁶Pb/²⁰⁴Pb and ²⁰⁷Pb/²⁰⁴Pb against ²⁰⁶Pb/²⁰⁴Pb diagrams (Fig. 2), the ratios for the ophiolitic sulphide deposits in Cyprus define trends extending from near the mid-ocean ridge basalt fields (such as Alestos) to within the seawater fields defined by analysis of the Pb isotopic composi-tion of manganese nodules^{12,13} (such as Mathiati). Values for three of the deposits (Skouriotissa, Peristerka and Limni) cluster in an intermediate position, whereas two sets of determinations for Mousoulos have the highest ²⁰⁶Pb/²⁰⁴Pb ratios but have slightly lower ²⁰⁷Pb/²⁰⁴Pb and ²⁰⁸Pb/²⁰⁴Pb ratios than the seawater fields. The ranges enclose the values for the epidote-sulphide veined metadolerite dyke sample (CY/75/71; no. 7 in Fig. 2), which has already been noted as possibly Pb isotope contaminated, and are also surprisingly similar to the Pb isotope ranges for Fe-Mn hydroxyoxide sediments (umbers) associated with the ophiolitic rocks of Cyprus and the Baër-Bassit area in Syria¹¹. These oxidized metalliferous sediments are also thought to have formed as chemical precipitates from submarine hot springs. As the Pb isotope ratios for the sulphides occupy positions intermediate between the mid-ocean ridge basalt fields, which also contain values for the ophiolitic mafic rocks in Cyprus, and the seawater fields, the sulphide deposit lead apparently does contain a basaltic component, as expected, but the lead is actually a mixture which also contains lead derived from seawater.

The isotopic evidence for mixed lead in the ophiolitic sulphide deposits of Cyprus differs from data recently obtained for sulphide samples from 21°N on the East Pacific Rise ^{14,15}. For example, Vidal and Clauer ¹⁴ report ²⁰⁶Pb/²⁰⁴Pb, ²⁰⁷Pb/²⁰⁴Pb and ²⁰⁸Pb/²⁰⁴Pb ratios of ~18.48, ~15.49 and ~37.90. These values fall well within the MORB Pb isotope fields (Fig. 2) and correspond closely with the averages of the Pb isotopic

Fig. 1 Locations of the volcanogenic sulphide ore deposits in the ophiolitic rocks of the Troodos Massif, Cyprus, for which Pb isotope ratios have been determined in this study and by Doe and Zartman (Skouriotissa)⁹.



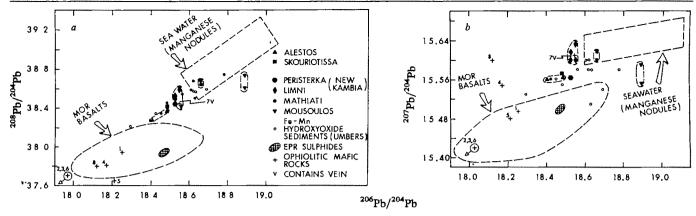


Fig. 2 Pb isotope ratios for volcanogenic sulphide ore deposits in the Troodos Complex, Cyprus, plotted together with ratios for a variety of variably hydrothermally metamorphosed mafic ophiolitic rocks from Cyprus, Fe-Mn hydroxyoxide rich chemical sediments (umbers) from Cyprus and Syria¹¹, general fields for mid-ocean ridge basalts¹⁰, general fields for seawater obtained by Pb isotopic analysis of manganese nodules^{12,13}, and sulphides from 21°N on the East Pacific Rise¹⁴. The numerals near the symbols for the ophiolitic mafic rocks refer to the samples listed in Table 1.

compositions of the associated basaltic rocks¹⁴. However, Fig. 2 shows that the lead isotope ratios for particular deposits in Cyprus seem to have restricted compositional ranges. In fact, the differences between ratios for Alestos and Mousoulos, both of which are in Cyprus, are actually greater than the differences between Alestos and the EPR 21°N samples. These sulphide deposit fields presumably, therefore, reflect a combination of the local lead isotope and mixing characteristics of each individual ore-forming geothermal system. The influence of source region is well shown by the more radiogenic Pb in the Kuroko volcanic sulphide ore deposits of Japan 16. 206 Pb/204 Pb, ²⁰⁷Pb/²⁰⁴Pb and ²⁰⁸Pb/²⁰⁴Pb ratios for galenas from 10 deposits of 18.42-18.62, 15.55-15.72 and 38.51-39.14 are interpreted to reflect variable lead derivation from Miocene volcanics and Palaeozoic basement¹⁶

One explanation of the differences in the Pb isotopic compositions of the EPR and Cyprus sulphides relates the facts that the EPR deposits are young and small. Hence, they are relatively enriched in unradiogenic leachable basaltic lead. The Cyprus deposits on the other hand, represent a completed process with a higher integrated water: rock ratio. The basaltic lead leached initially may have been diluted by more radiogenic seawater lead added subsequently.

We conclude that: (1) The Pb isotopic composition of the ophiolitic mafic rocks of the Troodos Complex is comparable with that of mid-ocean ridge basalts, but extends to such an unradiogenic combination of ²⁰⁶Pb/²⁰⁴Pb, ²⁰⁷Pb/²⁰⁴Pb and ²⁰⁸Pb/²⁰⁴Pb ratios as 16.620, 15.401 and 36.511 (T74). (2) The hypothesis of metal derivation by leaching of ophiolitic mafic rocks to form the volcanogenic sulphide ore deposits of Cyprus has some support. (3) The lead is, in fact, a mixture and seems to contain a variable component derived from seawater as well as a basaltic component. This observation differs from observations on recent sulphides from the East Pacific Rise, 21°N (refs 14,15). (4) Pb mixing probably occurred during convection within oceanic crust and not during discharge, as most of the samples examined were deliberately obtained from stockworks which were originally located at variable depths below the ocean floor. Data for a metadolerite sample containing originally dissolved Pb in a hydrothermal epidote-sulphide vein (CY/75/71) are consistent with this suggestion. (5) Lead in conformable sulphide deposits which has been used to construct lead isotope evolution curves (see refs 17,18) need not be exclusively of mantle derivation even when the associated igneous rocks are dominantly basaltic in composition as in the Troodos ophiolitic complex. Contamination by seawater may account for some of the scatter observed in detailed examinations of such conformable deposits (see ref. 19). The Pb isotope scatter is small, however, compared with the overall Pb isotope evolutionary range.

E.T.C.S. acknowledges receipt of operating grant A6114 from the Natural Sciences and Engineering Research Council of Canada and N. H. G. acknowledges partial support of this research by the NERC and by the SRC. We thank Roy Goodwin, Martin Humm (analysts), Sublash Shanblag (draftsman) and Brian O'Donovan (photographer) for invaluable assistance.

Received 2 October 1981, accepted 11 January 1982

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Temporal changes in the inorganic carbon system of the southeastern Bering Sea during spring 1980

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Previous studies of the inorganic carbon system in the southeastern Bering Sea1 demonstrated the occurrence of extremely low partial pressures of carbon dioxide (p_{CO_2}) and depressed total carbon dioxide concentrations (\(\Sigma CO_2\)) during late spring. To test the hypothesis that these conditions develop

during the spring phytoplankton bloom, and to provide biological production data that would be independent of the carbon-14 technique², we monitored the inorganic system in this region

intensively during spring 1980. We demonstrate here that there is a clear relationship between the changes in the inorganic carbon system and the spring phytoplankton bloom. Initially (March-early April), $p_{\rm CO_2}$ values in the surface waters were near or above the mean atmospheric content of 339 p.p.m., and $\Sigma {\rm CO_2}$ concentrations were ~2 mmol l⁻¹. By the end of the bloom, these variables were sometimes below 125 p.p.m. and 1.75 mmol l⁻¹ respectively, and the changes were correlated with nutrient decreases and dissolved oxygen and chlorophyll increases. At a mid-shelf station, it was possible to calculate net community organic carbon production from our data. The average rate between 23 April and 23 May was 3.6 g C m⁻² day⁻¹, in fair agreement with carbon-14-based primary production estimates.

Our p_{CO_2} data were taken continuously by sampling an air stream with an intake located near the T. G. Thompson's bow and a water stream taken from a depth of 3 m. The water stream was passed through an equilibrator3, and both the air and equilibrator gases were dried before analysis with a Beckman 315B IR analyser. Standards consisted of mixtures of CO2 and artificial air. Water stream results were corrected for a temperature rise (~0.5 °C) between the sea chest and the equilibrator and for an effect that arises when dissolved oxygen partial pressures are different than atmospheric. The p_{∞_2} data shown in Fig. 2 are for moist air. We determined ΣCO₂ (CO₂+ $H_2CO_3 + HCO_3^- + CO_3^{2-}$) by stripping acidified samples with nitrogen and drying the evolved gas before determination with a Beckman 215B IR analyser standardized with sodium carbonate solutions. Carbon-14 primary productivity rates were obtained from 8 h incubations in a deck incubator about 1.5 °C warmer than ambient surface water. Daily versus incubation period light observations were used to extrapolate daily rates from the incubation period results. The rest of our methods are described elsewhere4.

Figure 1 shows the PROBES (processes and resources of the Bering Sea shelf) line and the location of three quasi-stationary fronts that are normal features^{4,5,6}. Although tidal flows are substantial, mean flows in the PROBES region are only $\sim 1-5~\rm cm~s^{-1}$. There is evidence⁵ to suggest that the mean flow in the region between the middle and inner fronts is 1 cm s⁻¹, making this a favourable region for inferring biological rates from our $p_{\rm CO_2}$ and $\Sigma {\rm CO_2}$ time series.

Figure 2 shows the temporal evolution of the surface $p_{\rm CO_2}$ regime along the PROBES line in relation to the mean atmospheric value. Initial and final nitrate values are also indicated. These values do not show the presence of the middle front which is confined to the lower half of the water column⁶.

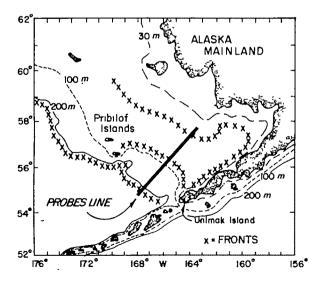


Fig. 1 Location chart showing the approximate position of the fronts that are present in the Bering Sea during the biologically active season, and the position of the main PROBES line.

As atmospheric exchange, vertical mixing, warming of surface waters as spring progressed and precipitation of calcium carbonate would all act to increase p_{CO_2} , the major factor causing the undersaturations seems to be the biological production of organic carbon. This hypothesis is supported by the nitrate data (Fig. 2) and also by the temporal changes in dissolved oxygen and chlorophyll. Initial (late March-early April) dissolved oxygen saturations in the surface waters were near 100%, but they rose as the bloom progressed. On the PROBES line, the maximum saturation value of 141% coincided with the minimum p_{CO_2} value of 120 p.p.m. (position 15 in Fig. 2, 16-17 March). Sometimes, maximum oxygen saturations occurred before minimum p_{CO_2} values. These situations might be expected because of the higher rate of O_2 exchange across the sea surface.

Initial chlorophyll values were generally less than $1 \mu g l^{-1}$ and never more than $1.5 \mu g l^{-1}$, but during the bloom, values in excess of $30 \mu g l^{-1}$ were encountered. Maximum chlorophyll values also occurred before minimum p_{CO_2} values in some cases. This is not surprising as the net productivity rate required to exceed our estimated atmospheric carbon dioxide invasion rates ($\sim 0.7 \text{ g C m}^{-2} \text{ day}^{-1}$, see below) is low in comparison with the maximum production rates.

Although biological production had the greatest effect on the p_{CO_2} values, physical processes also had observable effects. For example, the temporary reversal of the decreasing pw_{CO_2} trend at positions 11 and 12 on 22–23 April occurred during a period of enhanced vertical mixing.

As the p_{CO2} decreases (Fig. 2) seem to arise mainly from biological fixation of carbon, they should provide some insight into the timing of the spring bloom across the PROBES line. They suggest that some net productivity had occurred in the inner frontal region before our first observations. However, the first large p_{CO2} undersaturations occurred in or near the middle front and the bloom appears to have ended here and at position 15 first. By the end of our observational period (early June), the bloom appeared to be over everywhere, except for portions of the outer shelf and adjoining basin.

Some idea of the temporal variability in ΣCO_2 is given in Fig. 3. These changes, when combined with other data, allow the net community production rates to be estimated. We selected PROBES line position 12 for doing this because it is located in the region of weak mean currents mentioned above, and because we can compare the results with the relatively abundant carbon-14-based primary production measurements at this location. Salinity gradients in this region were weak, and using values that were normalized for salinity would not have changed the results. As the chemical results of biological production can be distributed by vertical mixing and advection, ΣCO_2 values were integrated over the total water column depth.

The interpretation of the changes in ΣCO_2 at position 12 is complicated by the existence of an anomalous station with high subsurface ΣCO₂ concentrations on 16 May. Examination of the temperature and nutrient distributions suggests that this was a transient introduced and removed by advection, but it significantly affects estimates of the ΣCO₂ removals arising from biological production. Regressions of $\Sigma CO_2 m^{-2}$ against time were 3.40 g C m⁻² day⁻¹ ($r^2 = 0.81$) with this value included and 4.32 g C m⁻² day⁻¹ ($r^2 = 0.94$) without it for the 23 April-23 May period. We believe that the latter regression is a better estimate of the change in ΣCO₂ concentrations at position 12 during this portion of the spring bloom as it minimizes random errors without being affected by the advective transient. That this transient influenced only a small portion of the shelf is suggested by similar regressions for the region between positions 11 and 13. Regressions with this value included and deleted gave rates of 4.12 g C m^{-2} day⁻¹ $(r^2 = 0.97)$ and $3.94 \text{ g C m}^{-2} \text{ day}^{-1} (r^2 = 0.97) \text{ respectively.}$

The above values have to be adjusted for the effects of mixing and advection, but only horizontal processes have to be considered as we are employing vertically integrated values. The existing transport models^{6,8} demand that a transient such as the

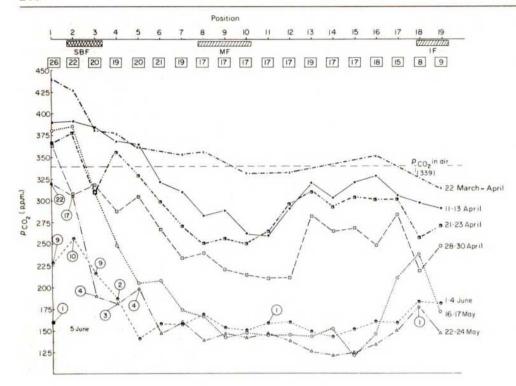


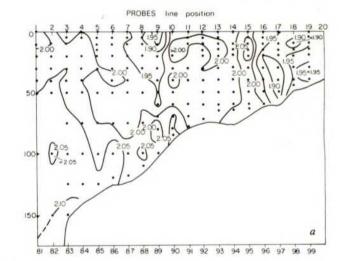
Fig. 2 The temporal evolution of p_{CO2} plotted against the mean atmospheric p_{CO_2} value along the main PROBES line during our 1980 experiment. The approximate locations of the fronts are indicated by the hatched boxes, and initial nitrate concentrations at the surface are indicated in the squares. Surface nitrate concentrations between 22 May and 5 June are shown in circles except for those cases where concentrations were less than 1 µg-atom 1-1. For clarity, partial lines taken on 2-5 and 6-9 May have not been included. Inclusion of these data would not have changed the general trends, but the number of temporary reversals in the generally decreasing trend would have increased. The pcO2 values are for moist air. On a dry air basis, the mean atmospheric value would be 341 p.p.m.

one observed at position 12 be averaged out over sufficiently long spatial or temporal domains, and it is not possible to do this using only the data for position 12. Hence, we used the position 11-13 space for this calculation (with the transient included). The above models suggest that longshore transport is insignificant, a conclusion that agrees with the longshore ΣCO₂ gradients that we observed. They also parametrically include mixing and advection in a cross-shelf diffusion coefficient which the most recent study suggests is 1× 106 cm2 s-1 in this domain (L.K. Coachman, personal communication). Applying this value to the appropriate gradients gives a diffusive supply of 0.14 g C m⁻² day⁻¹. Combining this value with the companion result from the regression analysis gives a total rate of 4.3 g C m⁻² day⁻¹. To test the strength of this calculation, we examined every possible combination of regression values and diffusive transports for position 12 only and for the position 11-13 region using calculations with and without the transient. This procedure had little effect on the answer for the position 11-13 region, the maximum difference being $0.3 \text{ g C m}^{-2} \text{ day}^{-1}$. For position 12 only, where it may not be appropriate to calculate the diffusive supply with the available model, the values ranged from 3.21 to 4.86 g C m⁻² day-1

With the appropriate constants and our temperature, salinity, p_{CO_2} and ΣCO_2 data, we calculated that approximately one-third of this decrease was due to the precipitation of calcium carbonate. This value seems reasonable, and if it applies to the entire water column, the decrease arising from the production of organic matter is 2.9 g C m⁻² day (based on our best estimate of 4.3 g C m⁻² day) for the total decrease).

A correction also has to be made for CO_2 invasion from the atmosphere. This was done by using the measured p_{CO_2} gradients across the air–sea interface and the 'piston velocity' concept^{7,10}. Literature values¹¹ were used to convert p_{CO_2} values into concentrations, and the average wind speed observed during the interval spanned by each gradient observation was used to obtain the appropriate 'piston velocity'. The average timeweighted invasion rate was $0.7 \text{ g C m}^{-2} \text{ day}^{-1}$ for both position 12 and the position 11–13 region. The net community organic carbon production rate arising from the above analysis is $3.6 \text{ g C m}^{-2} \text{ day}^{-1}$.

Seven carbon-14-based primary production rate determinations made at position 12 (between 20 April and 26 May) give



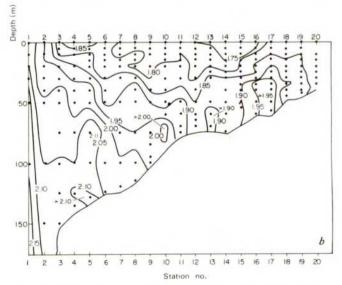


Fig. 3 Total carbon dioxide (in mmol l⁻¹) distribution on the PROBES line, 21-23 April (a) and 22-24 May (b).

a time-weighted average rate of 3.5 g C m⁻² day⁻¹ for the 23 April-23 May period. One would expect that the rate based on the changes in the inorganic carbon system should be smaller than the carbon-14-based rate. The difference between primary and community production could be small in the prevailing conditions, however, because the phytoplankton community at position 12 was composed of diatom species which did not seem to be significantly affected by herbivores that were present in low numbers. Although the carbon-14 method does not always compare favourably with other methods¹²⁻¹⁴, it does give reasonable estimates of particulate organic carbon production for some diatom populations¹² in eutrophic conditions^{15,16}. This discrepancy could also arise from errors in our comparison. Large errors in the choice of a diffusion coefficient, in the air-sea gas exchange, or in the amount of calcium carbonate precipitation would be required to alter our net community production rate by 1 g C m⁻² day⁻¹, but our test of our calculation suggests that factors such as insufficient resolution of temporal and

Received 28 August 1981; accepted 11 January 1982.

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spatial gradients could cause errors of this magnitude. An examination of the carbon-14 results suggests that this value may also be a partial function of sampling density. Because differences of the order of 1 g C m⁻² day⁻¹ could arise from errors associated with sampling density, there is no firm evidence to suggest that the carbon-14 method itself suffered from large errors in the conditions of our experiments. However, it is possible that this method estimated net community production more accurately than it estimated primary production.

We thank our colleagues in the PROBES program for data and comments, and G. Grunseich, A. Hafferty and D. Lowman for assistance. Financial support was provided by the NSF's Division of Polar Programs. We particularly thank Dr J. J. Kelly for advice and some necessary equipment. Complete listing of our data can be obtained from PROBES Data Management, c/o Dr H. J. Niebauer, IMS, University of Alaska, Fairbanks, Alaska 99701, USA.

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Variability in the abundance of animal and plant species

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A striking and consistent observation in ecology is that variability in population abundance of a species and average population density are related in both space and time1,2. Taylor and his colleagues have shown that this relationship between variance (V) and mean (M) conforms well to a simple power law for a very wide range of animal and plant species, with the logarithms of sample variance and average sample density following a linear relationship¹⁻³ ($V = \hat{a}M^{\delta}$ where \hat{a} and \hat{b} are constants). They suggest that the slope of this line, \hat{b} , which generally falls within the range 1 to 2, is a species-specific characteristic reflecting the balance between the opposing behavioural tendencies to aggregate within, and migrate from, centres of population density4. Taylor and co-workers1-6 have stressed that migration between patches is rarely a random process, on the evidence of the observed 'density-dependent' relationships between the degree of dispersion (the variance to mean ratio, V/M and mean abundance per patch). We show here that the relationship between variability and average abundance can be a simple, and inevitable, consequence of chance demographic events in the dynamics of population growth and decline. The precise form of these relationships is determined by the relative magnitude of the various rate processes which govern the dynamics of population change (the birth, death, immigration and emigration rates), and by the degree of spatial and temporal heterogeneity. We do not need to invoke any complex behavioural mechanisms to explain observed patterns.

Random or chance events play a major role in the population dynamics of all organisms. In any given time interval there is

only a certain probability that an organism will die or give birth, or that an immigrant will arrive or emigrant leave, partly as a consequence of the intrinsically discrete structure of populations7. The significance of such demographic stochasticity7-9 to observed patterns of variability and abundance can be examined using simple markovian population models. We begin by considering the temporal dynamics of a population in a defined space subject to constant rates of birth, death, emigration and immigration (denoted respectively by λ , μ , γ and δ) which are independent of population density (λ , μ and γ are defined as per capita rates while δ is defined per population). A continuous time stochastic model predicts that the probability distribution of population size at any point in time is overdispersed and negative binomial in form $^{10-12}$, where at time t (the initial population size N_0 at time t = 0 is taken to be zero), the mean M(t), and variance V(t), are given by

$$M(t) = \frac{\delta}{r} [e^{rt} + 1] \tag{1}$$

$$V(t) = M(t) \left[1 + \frac{\lambda}{\delta} M(t) \right]$$
 (2)

The intrinsic growth rate of the population, r, is defined as $r = (\lambda - \mu - \gamma)$. The variance to mean ratio, V(t)/M(t), is greater than unity and tends to rise linearly as average abundance increases (density dependent in the terminology of Taylor^{1,4}), with slope λ/δ . At low population densities, when the value of δ is large in relation to λ (often the case during the early stages of habitat colonization), the variance will be approximately equal to the mean. As average density rises, the log-log plot of variance against mean will become linear, with a maximum slope of 2 (Fig. 1a). The slope of the best-fit linear equation will therefore lie between 1 and 2 depending on the range of densities sampled and the relative magnitudes of the parameters λ and δ (Fig. 1a). Note that the vast majority of observed relationships also have slopes lying between 1 and 2

Simple stochastic models of pure population processes show how different patterns of dispersion can be generated. Birth rates, for example, generate over-dispersion, immigration rates generate randomness, while death and emigration rates tend create under-dispersion^{8,13,14}. Observed patterns

dispersion therefore depend on the dynamic balance between these opposing forces.

Stochastic models can also aid in the interpretation of spatial patterns of variability. For example, assume that a habitat is divided into P patches where the immigration rate per patch, δ , is random but dependent on the net rate of emigration from all other patches. When all other rates are constant and independent

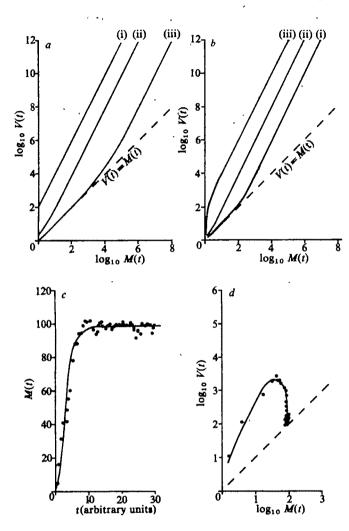


Fig. 1 a, The relationship between the logarithms of the variance in population size at time t, V(t), and mean population size, M(t), predicted by equations (1) and (2) in the text. The three solid lines denote the relationship for different birth (λ) and immigration (δ) rates: line (i), $\lambda/\delta = 0.001$; line (ii), $\lambda/\delta = 1$; line (iii), $\lambda/\delta = 100$. The probability distribution of population size is negative binomial in form parameter k (which varies inversely with the degree of dispersion) equal to δ/λ . The dashed line denotes the relationship for a Poisson distribution where V(t) = M(t), b, Similar to a but representing the relationship predicted by the spatial model defined in equations (3) and (4). The three solid lines denote the relationship for different values of the parameter combination C = [A+B]/[A-B] (see text): line (i), C = 0.01; line (ii), C = 1.0; line (iii), C = 100. Initial population size N_0 was set at 1. The dashed line is as defined for a. c, d Present the results of a Monte Carlo simulation experiment of the growth of a population subject to constant rates of birth, immigration and emigration, and a density-dependent rate of mortality $\mu(N)$, where $\mu(N) = \mu + \alpha N$ (N represents population density). The experiments were carried out along standard lines^{13,14} where the equivalent deterministic differential equation for the temporal dynamics of N is of the form $dN/dt = \delta + (\lambda - \gamma)N - (\mu + \alpha N)N$. a Records changes in the mean population size, M(t), through time, estimated from 25 replicate simulations. The solid dots are experimental results while the solid line is fitted by eye to denote the general temporal trend. In b the logarithms of the variances in population size (estimated from the replicate simulations) at time t, V(t), are plotted against the logarithms of means M(t). The dots are experimental results while the solid line is fitted by eye. The dashed line denotes the Poisson prediction where V(t) = M(t). Note that the relationship between $\log_{10} V(t)$ and $\log_{10} M(t)$ is curvilinear, where the degree of dispersion rises to a peak and then declines at high average densities: The parameter values used in the simulations were $\delta = 0.5$, $\lambda = 7.5$, $\mu = 2.0$, $\gamma = 0.5$ and $\alpha = 0.05$ (all defined per time unit).

dent of population density, the mean abundance per patch M(t), and the variance in density between patches, V(t), are now approximately given by

$$M(t) = N_0 \exp[(A-B)t]$$
 (3)

$$V(t) = \frac{(A+B)}{(A-B)}M(t)\left[\frac{M(t)}{N_0} - 1\right] \qquad (4)$$

The parameters A and B are defined for notational convenienc as $A = \delta P \gamma / (b + \delta P) + \lambda$ and $B = (\mu + \gamma)$; N_0 is the total popular tion size in the habitat at time t=0 and b is the per capit death rate of the migrating organisms. It is assumed in the derivation of equations (3) and (4) that there is a risk associate with migration such that $b \gg \mu$ and that in any given pate population growth is a simple birth (at a rate A)—death (at rate B) process where a component $(\delta P \gamma/(b + \delta P))$ of the birt rate is the arrival of immigrants from the remaining patches^{8,1} The probability distribution of abundance per patch is overdipersed in form (similar, but not identical, to the negative binomial distribution) where V/M increases linearly with mea abundance, with a slope of $(A+B)/[N_0(A-B)]$. The log-lc plot of variance against mean is again approximately linea with a slope in general lying between 1 and 2, depending o the range of densities sampled and the values of the populatic parameters (Fig. 1b). (At very low average densities the slot may even be greater than 2.)

This result does not support Taylor's view (see above) the such patterns are likely to be due to nonrandom migratio because our simple model assumes migration to be a randor process between patches. Similar conclusions have also bee reached by Hanski¹⁵.

We have so far assumed that the rates of population chang are independent of density; of course, this is rarely the case natural habitats. Density dependence, whether acting on the birth, death, emigration or immigration rates (or any combination) tion of these), serves to regulate population abundance in given habitat to a typical equilibrium K (refs 16-18). Mar factors may account for such density dependence, includir complex behavioural processes (which often influence emigra tion and/or immigration rates). When a population is at or ne K, density-dependent processes (which reduce birth ar immigration rates, or increase death and emigration rates, density rises) tend to generate underdispersion, as illustrate by the Monte Carlo simulation experiments recorded in Fi 1c, d. The pattern of dispersion changes from random whe the habitat is being colonized, to overdispersed as the popul tion grows exponentially, to random or underdispersed as th population approaches K. A curvilinear relationship betwee $\log V$ and $\log M$ is thus produced, as shown in Fig. 1d.

Patterns of this form are seldom recorded even for 'I strategist'¹⁹ species living in relatively stable habitats. Or example is provided by the vole, *Microtis pennsylvanicus*, in i grassland habitat, where the degree of dispersion changes from overdispersed to underdispersed as population densitincreases²⁰. In the case of 'r-strategists'¹⁹ we are unaware any examples similar to that in Fig. 1d. Thus, contrary to the predictions of our model, there is little evidence that the degree of dispersion declines at high population densities¹⁻⁴.

The models examined so far, however, fail to take accound a crucial feature of almost all natural habitats, namely environmental heterogeneity. Because of fluctuating environmental conditions, both in space and time, population rate parameters are in reality random variables, not constants 21,2 . Environmental stochasticity acts to compound the underlying variability created by demographic events, and has a centrarole in determining observed patterns of population dispersion. To illustrate this, we consider a model in which the carrying capacity, K, of each patch is a random variable (normal distributed with mean K and variance s_K^2). Monte, Carls simulation studies reveal that log-log plots of the variance abundance between patches against the mean population density will tend to be approximately linear over all densities

with a slope lying between 1 and 2, provided the habitat is moderately heterogeneous. There is little evidence of a decline in the degree of dispersion at high average densities, despite the fact that density-dependent processes are regulating overall abundance (Fig. 3a, b, e, f). When sampling is restricted to patches where the density is near to the average carrying capacity, \vec{K} , then the slope of the log-log plot of variance against mean ranges from close to, or less than, unity if the habitat is relatively homogeneous, to greater than 2 if the habitat is highly heterogeneous (Fig. 3). Such slopes greater than 2 have occasionally been observed in studies of spatial and temporal trends in the pattern of population dispersion (Fig. 2).

The range of values over which the slope can vary is small (roughly 0.5-2.5, Fig. 2), its precise value being critically dependent on sample size, the range of densities over which samples are collected and the degree of environmental heterogeneity at the time and place of sampling (Fig. 3). For a fixed set of parameter values, for example, the slope may vary greatly between one simulation and another even when sample size is large. The slope will tend, on average, to be determined by the values of the population parameters that control the growth of

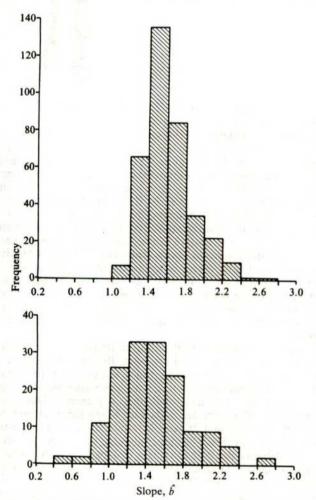


Fig. 2 Frequency distributions of the slopes of the best-fit linear model to the logarithms of sample variance and mean density estimated from a wide range of ecological data. The linear model is of the form $\log_{10}V = \log_{10}a + b \log_{10}M$ where b is the slope and $\log_{10}a$ the intercept b. Temporal trends. This figure denotes the frequency distribution of slopes b estimated from samples of the abundances of various moth and aphid species collected over a period of at least b yr at sites in Great Britain and mainland Europe (data analysed by Taylor and Woiwood²). The means and variances in population abundance from which the slopes were estimated were calculated from samples taken at one site over a period of 1 yr. The frequency distribution represents slopes for 360 species. b, Spatial trends. Similar to a but representing the frequency distribution of slopes estimated from samples of the abundances of species ranging from plants, through protozoa, to mammals, taken at one point in time at different spatial sampling sites (data analysed by Taylor, Woiwood and Perry¹). The frequency distribution records slopes for 155 species.

a given species, but these often vary on a regular seasonal basis. It may be possible, however, to discern general trends in the sense that species with high birth rates that live in unstable heterogeneous habitats (r-strategists) will tend to have steeper slopes than those that have low birth rates and live in stable

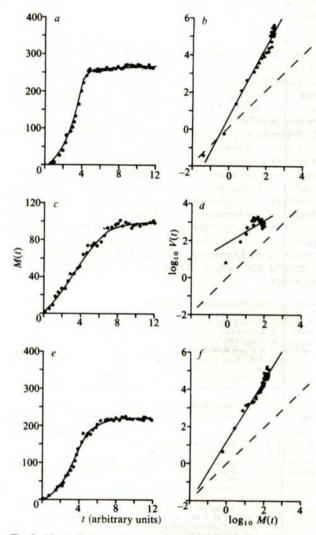


Fig. 3 Monte Carlo simulation studies of the impact of environmental stochasticity on population growth and the degree of dispersion. The results presented represent three experiments using a model in which the carrying capacity of the patches within a habitat is assumed to be a random variable. The model is identical to that described in the Fig. 1 legend to (c, d) except that the density-dependent coefficient, α , was assumed to be normally distributed with mean $\bar{\alpha}$ and variance s_{α}^2 . The simulations were carried out along standard lines ^{13,14} except that for each replicate of population growth within a patch a pseudorandom number was used to select a value for α and hence determine the carrying capacity, K, of the patch. K was assumed to be constant through time for any given patch. a, b Portray the results of one experiment in which the parameter values were set at $\delta = 0.5$, $\lambda = 7.5$, $\mu = 2.0, \ \gamma = 0.4, \ \bar{\alpha} = 0.05, \ s_{\alpha}^2$ $\mu = 2.0$, $\gamma = 0.4$, $\bar{\alpha} = 0.05$, $s_a^2 = 0.025$. a Records changes in the mean population density per patch, M(t), through time, of 25 patches. The solid dots denote experimental results while the solid line is fitted by eye. b Records the relationship between the logarithms of the variance in population size between patches at time t, V(t) (given that each patch has a different carrying capacity, K, where

$$K = [\lambda - (\gamma + \mu) + [(\gamma + \mu - \lambda)^2 + 4\alpha\delta]^{1/2}]/(2\alpha))$$

and the mean population size per patch, M(t) (sample size = 25). The solid dots denote experimental results while the solid line represents the best-fit linear model of the form $\log_{10}V(t) = \hat{a} + \hat{b} \log_{10}M(t)$, where the constants have the values $\hat{a} = 0.76$, $\hat{b} = 1.91$. The dashed lines denote the Poisson prediction where V(t) = M(t). c, d Are similar to a and b except that in the simulation experiments (25 replicates) the variance of the random variable α is set at $s_a^2 = 0.0125$ to represent a fairly homogeneous habitat (in the experiments recorded in a and b the larger value of s_a^2 implies a more heterogeneous habitat). In d the parameters of the best-fit linear model are $\hat{a} = 1.87$, $\hat{b} = 0.58$. e, f Are again similar to a and b except that the parameters λ and μ were changed to $\lambda = 10.0$ and $\mu = 5.0$. The degree of environmental heterogeneity was the same as that for a and b. In f the parameters of the best-fit linear model are $\hat{a} = 1.11$, $\hat{b} = 1.69$.

and relatively homogeneous habitats (K-strategists). Some support for this conjecture is provided by the available empirical

The patterns of dispersion generated by simple models of population growth lead us to believe that observed relationships between variability in population abundance and average density, highlighted by the work of Taylor³, are in general a simple and inevitable consequence of demographic and environmental stochasticity. It is not necessary to invoke explanations based on the behavioural tendencies of species to aggregate and migrate4 in order to understand the trends that have been observed in natural habitats. Indeed, in a spatially uniform world, and in the complete absence of demographic stochasticity, such tendencies will not generate power law relationships between population variability (V) and average abundance (M). When density-dependent factors are of limited significance (r-strategists) demographic stochasticity alone is sufficient to account for the approximately linear relationship between the logarithms of variance and mean abundance and for the slopes of such relationships lying on average between 1 and 2 (Fig. 2). In the presence of strong density dependence (K-strategists), a degree of environmental heterogeneity (either in space or time, or both) will ensure that such relationships remain approximately linear over all average densities (Fig. 3)

We thank M. Loevinson, J. Soberon, J. Lawton and T. R. E. Southwood for helpful discussions and comments on the manuscript.

Received 16 October 1981, accepted 28 January 1982.

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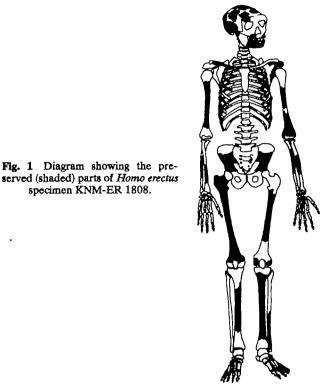
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A possible case of hypervitaminosis A in *Homo erectus*

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Following an initial discovery by Bw. K. Kimeu in 1973, sleving operations have recovered the most complete Homo erectus skeleton so far known (Fig. 1) from the Upper Member of the Koobi Fora Formation in Area 103, Koobi Fora, East Lake Turkana in Kenya^{1,2}. The partial skeleton shows pathological changes consistent with chronic hypervitaminosis A. We attribute this disorder to the high dietary intake of animal liver, most probably that of carnivores, during a period when the dietary habits of Homo erectus were changing.



The geological age of the specimen (KNM-ER 1808) is constrained by the ages of the overlying Koobi Fora Tuff and the top of the underlying Olduvai Event. The Koobi Fora Tuff is actually a complex body of tuff, tuffaceous sediment and sediment, as are the Okote and Middle Ileret Tuffs. Cerling and Brown (personal communication) have established that some parts of each of these tuff complexes can be correlated. Fitch and Miller³ reported K/Ar dates on sanidine separates from the Koobi Fora Tuff ranging from 0.53 to 4.44 Myr, dates on the Okote tuff ranging from 0.87 to 1.70 Myr, and a date of 1.48 Myr for the Middle Ileret Tuff. Their preferred ages for the Koobi Fora and Okote Tuffs are 1.57 and 1.56 Myr respectively. Curtis (personal communication) has dated the Okote Tuff at 1.48, 1.46 and 1.44 Myr. Thus a reasonable younger limit on the age of the specimen is 1.5 Myr. The age of the top of the Olduvai Event is given as 1.76 Myr by MacDougall⁴, providing an older limit for the age of the specimen.

Fig. 1 Diagram showing the pre-

specimen KNM-ER 1808.

The precise position of the polarity transition is uncertain, but is known to lie at least 12 m below the specimen, and may lie much lower in the section. Since the specimen lies fairly close to the Koobi Fora Tuff, a reasonable estimate of its age is 1.6 ± 0.1 Myr. This adult individual is presumed female by comparison of innominate parts with KNM-ER 3228 (ref. 5) and Olduvai Hominid 28 (ref. 6), which are considered male and female respectively by modern human osteological criteria.

The appendicular skeleton shows striking pathology, consisting of subperiosteal diaphyseal deposit of coarse-woven bone. The new bone, 7.0 mm thick in places, thins towards the metaphyses. There is minimal endocranial involvement. Ground thin sections of the tibial shaft (Fig. 2) show pathology confined to the outermost cortex, which has given rise locally to new bone. The sharply demarcated, coarse-woven new bone contains enlarged, sub-spherical and randomly placed lacunae. There is no evidence of abnormal remodelling of the underlying bone. The dense mineralization of the specimen accounts for the fine histological preservation, but precludes satisfactory radiological examination.

Although a disease that no longer exists or has changed its manifestations can neither be diagnosed nor excluded, we suggest that KNM-ER 1808 had chronic hypervitaminosis A. Although the night blindness of vitamin A deficiency has been known since antiquity, the deleterious effects of excessive ingestion became known only much more recently. Early polar explorers reported the development of an acute toxic state on ingestion of polar bear, seal or husky dog liver^{7,8}. However, it was not until 1942 that Rodahl and Moore⁹ identified vitamin A as the toxic component of seal and polar bear liver.

Acute hypervitaminosis A, characterized by symptoms of peeling of the skin, vomiting and diarrhoea, headache and convulsions, may be fatal. The condition, now rarely seen in adults, is fairly common in infants after accidental ingestion or misguided parental enthusiasm¹⁰.

Chronic hypervitaminosis A is a considerably more subtle disease, which became recognized as a clinical entity only in the mid-twentieth century. Although the condition is fairly common in children¹¹⁻¹⁴, only 17 cases have been reported in adults since 1951^{7,11,15-22}.

Skeletal manifestations have been variable in these patients. Periosteal calcification of the long bones, well known in children²³, was detected in only 3 of the 17 adults^{7,17,18}. The only case biopsied⁷ showed thickened tibial periosteum with the formation of poorly calcified coarse-fibred new bone. All cases of hypervitaminosis A are probably associated with bone changes, although standard radiographic techniques are insensitive to early changes²⁴. Another case¹⁹, biopsied in the absence of radiological abnormalities, showed large osteocytic lacunae, focal hypermineralization and an increased rate of bone turnover. The enlarged lacunae are thought to be due to the osteocytes assuming an osteoclast-like function, accounting for the hypercalcaemia of hypervitaminosis A.

Bone changes are seen in adult experimental animals with administration of excess amounts of vitamin A^{25,26}. Rats fed large doses of vitamin A showed considerable periosteal bone deposition, with low density, cancellous-type new bone overlying the cortical bone²⁷. Large osteocytes, seen within confluent lacunae, matured rapidly, tending to produce osteolysis with partial breakdown of the bone matrix.

Other conditions causing abnormal calcification must be considered in a differential diagnosis. Hypervitaminosis D leads to hypercalcaemia and metastatic visceral calcification, with the bones becoming markedly osteoporotic. Cystic lesions of the bones ('brown tumours') are seen in hypoparathyroidism. Some modern cases of hypoparathyroidism have shown increased cortical density, but it is unlikely that a hominid living 1.6 Myr ago would have survived the associated tetany long enough to have developed skeletal changes. Fluorosis also produces osteosclerosis, but this is said to be due to both periosteal and endosteal bone formation. The histology is not well documented^{28,29}. Subperiosteal calcification in scurvy, usually metaphyseal rather than diaphyseal, is associated with cortical thinning and epiphyseal separation28. Infantile cortical hyperostosis involves the mandible and resorbs with ageing30. Generalized cortical hyperostosis (Van Buchem's disease) affects many parts of the skeleton, but shows endosteal thickening, especially in the skull³⁰. The periosteal bone deposition of osteomyelitis variolosa, associated with childhood smallpox, is most prominent near the joint capsule, with possible sequestration of the epiphysis³¹⁻³³. In syphilitic osteomyelitis, the skull is almost always involved; endosteal proliferation is usual, as are large destructive foci (gummas)^{30,31}. Hypertrophic osteoarthropathy is often associated with periosteal bone deposition, but the histology is that of normal cortical bone31

The most likely diagnosis is, therefore, hypervitaminosis A. If we are correct, how did this *Homo erectus* ingest such large doses of the vitamin?

In the Koobi Fora succession, the first record of the use of stone artefacts and their association with animal bones, presumably representing early hominid meals, antedates KNM-ER 1808 by some 200,000 yr. There was probably a major change in the diet of early humans, with a large increase in meat eating, at that period and it may have taken some time to learn which parts of which carcasses were poisonous. One hundred grammes of modern herbivore liver contain 44,000-50,000 IU of vitamin A, whereas 100 g of carnivore liver contain 1.3-1.8 × 10⁶ IU as carnivores derive and store large amounts of preformed vitamin from livers of their prey. The condition described above is

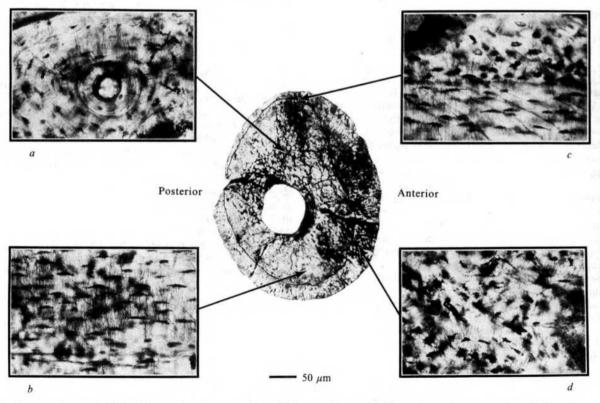


Fig. 2 Cross-section at midshaft of femur showing outer layer of abnormal bone and thin sections taken approximately from the positions shown. a, Normal osteon from deeper part of shaft. b, Outer lamellar bone showing normal, flattened osteocytic lacunae and canaliculi. c, Junction of normal lamellar and abnormal bone, showing sudden transition from normal lacunae to subspherical, disorganized lacunae with more randomly disposed canaliculi. d, Disorganized area typical of the abnormal bone.

unlikely to be due to ingestion of the easily masticated liver of a herbivore (there is no evidence of the use of fire at this time), but quite possibly to a diet containing carnivore liver. Interference behaviour of the type seen today between carnivore species³⁴ would be expected when early hominids began to compete directly with carnivores. Some of these interactions might have resulted in carnivores being killed and eaten by hominids.

Alternatively, or additionally, selection for tolerance to high levels of vitamin A may have been slow, with KNM-ER 1808 representing one individual who could not store vitamin A at high levels. This is unlikely, however, because normal human liver levels are within herbivore ranges³⁵. It is more likely that

Received 14 July 1981: accented 25 January 1982

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early humans learned to avoid carnivore liver rather than were selected for new physiological limits.

We thank the Museum Trustees of Kenya, Bw. K. Kimeu, who made the initial discovery and directed the sieving operations, and the following for valuable discussions and help: J. Bieri, J. W. Bowerman, F. H. Brown, T. E. Cerling, J. B. Cohn, G. H. Curtis, M. W. Donner, J. P. Dorst, M. T. Freedman, M. J. Glincher, M. G. Leakey, A. L. Lehninger, O. Lovejoy, L. Perez, S. S. Siegelman and B. Van Valkenburgh. We acknowledge support from the National Geographic Society and the Kenya National Museums to R.E.F.L. and NSF grants BNS 75-16879 and 78-24499 to A.W.

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β -Endorphin is processed differently in specific regions of rat pituitary and brain

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Recent studies have shown that β -endorphin is found in the pituitary with two related peptides, the C-fragment (β -endorphin₁₋₂₇)^{1,2} and the des-histidine derivative of the C-fragment $(\beta$ -endorphin₁₋₂₆)³, and that each of these peptides (Fig. 1) occurs also in an α , N-acetyl form^{3,4}. The six peptides are derived from a single precursor by differential proteolytic cleavage and acetylation. The fact that different forms of β -endorphin predominate in different regions of the pituitary5,6 implies that there are region-specific differences in processing of the precursor. Because β -endorphin is known to occur widely in the brain^{7,8} as well as in the pituitary we have now investigated the distribution of the six peptides in selected regions of rat brain. We report here that, as in the pituitary, different regions of the brain contain strikingly different proportions of the six β endorphin-related peptides, apparently as a result of differential processing.

Dissected regions of rat pituitary (10 male Sprague-Dawley rats, 250 g) and of rat brain (30 rats), and also whole brain (5 rats), were extracted in acetone/HCl/water (40:1:5, v/v) and the soluble products fractionated by gel filtration on a Sephadex G75 column (70×1.5 cm) in 50% acetic acid. The elution position of the group of β -endorphin-related peptides was identified using ¹²⁵I-labelled bovine markers added to each homogenate. The marker peptides, isolated from ox pituitary, were β -endorphin, α , N-acetyl β -endorphin, the C'-fragment and α , N-acetyl C'-fragment. The endogenous β -endorphinrelated peptides, together with the markers, were resolved by chromatography on a sulphopropyl Sephadex C25 (pyridinium form) column (70×0.6 cm) in 50% acetic acid with a 0-1 M pyridine linear gradient (100 ml mixer) and located by radioimmunoassay using an antibody raised against β -endorphin¹⁰. The identity of four of the six peptides was finally confirmed by HPLC on a C₁₈ microbondapak column in 0.01 M hydrochloric acid with a 25-40% acetonitrile linear gradient (30 min)⁵. The elution positions of the immunoreactive peptides were compared with the positions of the corresponding bovine peptides in consecutive chromatograms. Each experiment was repeated several times (five times for regional distribution of the pituitary and two to six times for region of brain); only minor differences were observed in the patterns of distribution and the amounts of each peptide.

In rat pars intermedia six peptides having β -endorphin immunoreactivity were resolved (Fig. 2); four corresponded to the known marker peptides, β -endorphin₁₋₃₁ (VI), acetyl β endorphin₁₋₃₁ (V), β -endorphin₁₋₂₇ (IV) and acetyl β -endorphin₁₋₂₇ (II), and the remaining components (III and I), which were the principal peptides of the pars intermedia, had chromatographic properties corresponding to porcine β -endorphin₁₋₂₆ and its α , N-acetyl derivative. Thus in rat pars intermedia, β -endorphin is a minor component and the major β endorphin-related peptides are essentially inert as opiates. In contrast, the major peptide in the anterior pituitary with the approximate molecular size of β -endorphin eluted in the position of β -endorphin₁₋₃₁. Similar processing patterns have

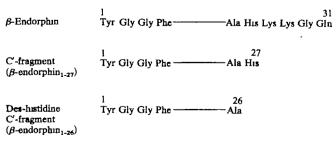


Fig. 1 β-Endorphin-related peptides identified in porcine pituitary. In the rat, valine replaces alanine at position 26 (ref. 11).

been found in other species, including pig^{3,5}, mouse, cat and guinea pig (S.Z. and D.G.S., unpublished results).

The six peptides identified in rat pituitary were shown to be present also in rat brain. Ion-exchange chromatography of the β -endorphin-related peptides extracted from whole brain (Fig. 3) indicated that the acetylated forms were relatively minor; the principal peptides were β -endorphin₁₋₃₁ and β -endorphin₁₋₂₆. Examination of the peptides extracted from regions of brain, however, revealed two contrasting processing patterns, one for the hypothalamus, midbrain and amygdala, and the other for the hippocampus, dorsal colliculae and brain stem. The hypothalamus contained predominantly the biologically potent form of β -endorphin and the midbrain and amygdala again contained β -endorphin but it was accompanied by β -endorphin₁₋₂₆ (Fig. 4a); in these regions there were negligible acetyl peptides. In contrast the hippocampus, dorsal colliculae and the brain stem also contained the α , N-acetyl forms of

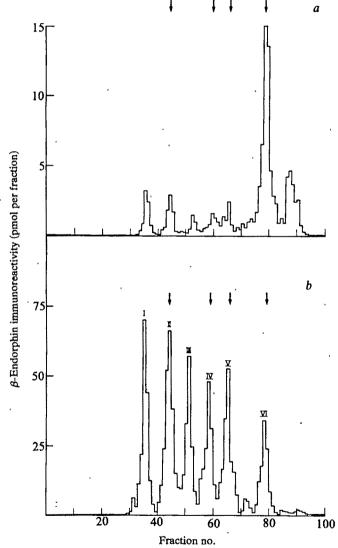


Fig. 2 Ion-exchange chromatography of β -endorphin-related peptides extracted from the anterior pituitary (a) and pars intermedia plus posterior pituitary (b) of the rat. The weights (in mg) of freshly dissected tissues were: anterior pituitary, 8.9 ± 1.2 ; pars intermedia, 1.1 ± 0.1 (mean \pm s.d. calculated from 20 rats). The elution positions of the radio-iodinated reference peptides $(\alpha, N$ -acetyl β -endorphin₁₋₂₇, β -endorphin₁₋₃₁), which exhibit slightly greater retention than the endogenous peptides, are indicated respectively from left to right by the arrows. α, N -acetyl β -endorphin₁₋₂₆ (peak I) and β -endorphin₁₋₂₆ (Peak III) chromatographed in positions not indicated by the marker peptides. The column conditions are described in the text. Fractions were 1.5 ml.

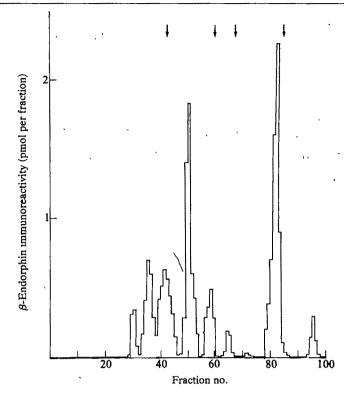


Fig. 3 Ion-exchange chromatography of β -endorphin-related peptides extracted from whole rat brain. The mean weight of intact brain was 1.83 ± 0.17 g (20 rats). The elution positions of the 125 I-labelled reference peptides $(\alpha,N$ -acetyl β -endorphin₁₋₂₇, β -endorphin₁₋₂₇, α,N -acetyl β -endorphin₁₋₃₁ and β -endorphin₁₋₃₁) are indicated from left to right by the arrows. α,N -acetyl β -endorphin₁₋₂₆ and β -endorphin₁₋₂₆ to not elute in positions indicated by the marker peptides. Note that the major peptides are β -endorphin₁₋₃₁ and β -endorphin₁₋₂₆. Fractions were 1.5 ml.

 β -endorphin₁₋₂₇ and β -endorphin₁₋₂₆; the NH₂ forms of the peptides were less significant (Fig. 4b).

These results point to the existence of differential processing mechanisms in various regions of rat brain, as is the case in different regions of the pituitary. In the anterior pituitary and hypothalamus, β -endorphin seems to be formed by the action of an enzyme that cleaves between lysylarginine and tyrosine (positions 39-41 of rat lipotropin¹¹). In the amygdala and midbrain, however, β-endorphin₁₋₂₆ is an additional component, which would seem to require the action of two further enzymes, an endopeptidase for cleavage at Lys-Lys-Gly (positions 68-70 of rat lipotropin) and a carboxypeptidase B-like enzyme for removal of C-terminal lysine and histidine. On the other hand, in the pars intermedia, hippocampus, dorsal colliculae and brain stem the most abundant peptides are derivatives of either β -endorphin₁₋₂₇ or β -endorphin₁₋₂₆ and they are largely present in the acetylated form. The elaboration of these peptides would seem to involve the action of at least four enzymes—the two endopeptidases, the carboxypeptidase B-like exopeptidase and an α , N-acetylating enzyme. Note that of the six peptides related to β -endorphin, β -endorphin alone possesses potent analgesic properties^{12,13} and the formation of any one of the other peptides is accompanied by almost complete loss of the analgesic activity3,14.

The secondary processing of β -endorphin may involve reactions that are time-dependent. In the brain, the processing could occur in the cell bodies where β -endorphin seems to be synthesized, during transport along axons, or during storage at the nerve terminals. Thus the peptides present in the different regions might reflect the duration of their exposure to specific processing enzymes. Indeed, the predominance of β -endorphin₁₋₂₆ and β -endorphin₁₋₂₇ rather than β -endorphin₁₋₃₁ in the brain stem, dorsal colliculae and hippocampus would be

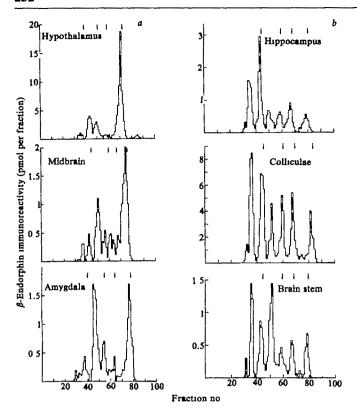


Fig. 4 Ion-exchange chromatography of β -endorphin-related peptides extracted from dissected regions of rat brain. The mean weights (in mg) of the freshly dissected tissues were: hypothalamus, 102 ± 18 ; midbrain, 131 ± 15 ; amygdala, 60 ± 19 ; hippocampus, 153 ± 22 ; dorsal colliculae, 127 ± 11 ; brain stem, 270 ± 30 (values calculated from 30 rats). The elution positions of the ¹²⁵I-labelled reference peptides (α, N-acetyl β-endorphin₁₋₂₇, β-endorphin₁₋₂₇, α , N-acetyl β -endorphin₁₋₃₁ and β -endorphin₁₋₃₁) are indicated from left to right by the arrows. Fractions were 1.6 ml. Note that the hypothalamus, midbrain and amygdala exhibit one pattern of processing (a) while the hippocampus, dorsal colliculae and brain stem exhibit another (b),

consistent with the concept that most of the secondary processing takes place distally from the hypothalamus, as it is only in the hypothalamus that cell bodies occur^{10,15}. On balance, however, the specific nature of the products formed in the different tissues, and the multiplicity of the enzymes necessary for their formation, suggests that distance from the site of biosynthesis is unlikely to be responsible for the organized distribution of peptides observed. The nature and amounts of the peptides present in each region are probably determined by the controlled action of specific enzymes located in those regions.

We thank Kamela Maruthainar for technical assistance.

Received 7 September 1981, accepted 18 January 1982.

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Insulin-like growth factor I stimulates growth in hypophysectomized rats

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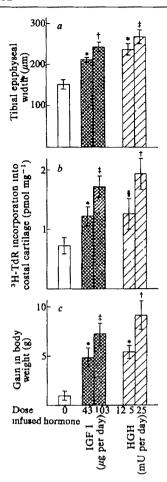
Although growth hormone stimulates the growth of hypophysectomized rats, it has long been proposed 1,2 that the effects are not direct but instead are mediated by the somatomedin peptides. Two of these are insulin-like growth factor I (IGF I) and insulin-like growth factor II (IGF II)3, so called because they are closely related to insulin in structure^{4,5}. IGF I and IGF II have somatomedin activity in vitro 6,7 but until now insufficient amounts of the peptides have been available to test their in vivo activity. We now report that pure IGF I stimulates the growth of hypophysectomized rats in a dose-dependent manner. This strongly supports the notion that the action of growth hormone is mediated by peptides of the somatomedin family.

The in vitro effects of insulin-like growth factors have been extensively studied. Both IGF I and IGF II are potent stimulators of cell replication, and of DNA and RNA synthesis in fibroblasts⁷, chondrocytes³ and calvarian cells (ref. 8 and C. Schmid and E.R.F., in preparation). Moreover, they mimic the effects of insulin on insulin target tissues such as adipose tissue⁷ and muscle⁹.

The recent development of specific antibodies against IGF I (ref. 6) and somatomedin C (ref. 10) have made it possible to investigate in man the dependence of these factors on the growth hormone (GH) status in various pathophysiological situations. Available evidence indicates that immunoreactive IGF I and somatomedin C are identical¹¹⁻¹³. The following clinical findings support the somatomedin concept²: immunoreactive IGF I is decreased in hypopituitarism and increased in acromegaly⁶. Treatment of hypopituitary children with growth hormone increases the serum immunoreactive IGF I levels¹⁴. Similarly, treatment of hypophysectomized rats with growth hormone causes a rise in their decreased serum IGF level¹⁵. Although clinical findings and in vitro results support the notion that IGF rather than growth hormone itself is the true growth stimulating hormone, there is no unequivocal direct evidence for this. There have been recent reports 16,17 that plasma peptide fractions containing somatomedin activity caused an increase in body weight and sulphate-incorporating activity of cartilage in Snell dwarf mice when administered subcutaneously three times per day for 2-4 weeks. Whereas van Buul-Offers et al. 16 also found an increase in nose-to-tail length, Holder et al. 17 observed no such effect. To prevent hypoglycaemia in the animals after bolus injections of the somatomedin preparation, van Buul-Offers et al.16 had to add 10% glucose to the drinking water. The latter problem was circumvented by Ellis et al. 18, who demonstrated that continuous subcutaneous (s.c.) infusion of a partially purified (4% purity) IGF preparation stimulated growth of hypophysectomized rats. The latter, in contrast to Snell dwarf mice, do not grow under thyroxine treatment, and therefore appear to represent a more specific experimental model for testing the somatomedin hypothesis. Therefore in this study we used hypophysectomized rats.

Male Tif RAI rats (100-120 g) were used for all experiments. Hypophysectomy was carried out 3-5 weeks before starting the experiments. Only those rats whose body weight did not increase by >0.2 g per day were considered to be totally hypophysectomized. The rats had free access to food (Altromin; Lage, FRG) and drinking water, and were kept on a 12-h light and dark cycle. Two doses of pure IGFI (43 and 103 µg per day)

Fig. 1 Stimulation of various growth indices by administration in vivo of IGF I and HGH to hypophysectomized rats (a = tibial epiphyseal width, thymidine incorporation into rib cartilage; c = daily body weight gain) Hormones were administered to groups of four rats for 6 days by continuous minipumps. IGF I was dissolved in 0 1 M acetic acid (43 or 103 μg per day); HGH (12.4 mU or 25 mU per day) (2 U mg⁻¹); Nordisk (Nanormon Insulin Laboratory, Denmark) was dissolved in saline. No difference was found between control rats treated for the same period with 0 1 M acetic acid or saline or left untreated. . Control hypophysectomized rats; II, IGF Itreated hypophysectomized rats; E, HGH-treated hypophysectomized rats. Each bar represents the mean \pm s.d. (n=4). *P<0.001; §P<0.01 compared with control (Student's *t*-test). $\dagger P < 0.01$, $\ddagger P < 0.05$ compared with the lower dose of hormone (Student's



were administered continuously for 6 days via s.c. implanted Alzet minipumps (no. 2001; Alza). The pumps (mean pumping rate $1.02\pm0.15\,\mu\text{l}\,\text{h}^{-1}$, mean filling volume $219.2\pm8.7\,\mu\text{l}$), were implanted under light ether anaesthesia. The pure IGF I preparation used was the same as that isolated and sequenced elsewhere4; its specific biological activity, as determined in the rat fat pad assay, was 330 mU mg⁻¹ (ref. 7). Pure IGF I was dissolved in 0.1 M acetic acid.

The body weight of the rats was measured daily at 08.00 h and they were killed after 6 days. The two lowest ribs were removed and ³H-thymidine (³H-TdR) incorporation into DNA was determined as follows: segments of cartilage were prepared free of perichondrial tissue and incubated (24 h, 37 °C) in Krebs-Ringer-phosphate buffer containing human serum albumin (2 mg ml⁻¹), nonessential amino acids (1% v/v; 5832 Difco), amino acids HeLA (1% v/v; 5790 Difco), glycine (0.2 mM), glutamine (2 mM) and ³H-TdR (1 μM). After incubation the cartilages were dried and weighed, digested in concentrated (90%) formic acid (30 min, 90 °C), then counted for radioactivity in 10 ml Instagel (Packard) in a β counter. The tibia test was carried out according to Greenspan et al. 19. The concentration of IGF I was determined by radioimmunoassay (RIA) before the minipumps were filled and after they were removed. The IGF I concentration was $1.88~\mu g~\mu l^{-1}$ in one group of rats before and $1.90~\mu g~\mu l^{-1}$ after 6 days of infusion. Thus, the IGF I concentration did not decrease in the implanted minipumps during 6 days under the skin.

Blood was sampled by aortic puncture and immunoreactive IGF I in pooled serum was determined by RIA. IGF I concentrations were 168 ng ml⁻¹ and 286 ng ml⁻¹, respectively, in the two IGF I-infused groups of rats. No immunoreactive IGF I was detectable in control and growth hormone-treated rats. The endogenous rat IGF, which increases after growth hormone treatment¹⁵, does not cross-react in the RIA with human IGF I. The lower dose of IGF I (43 μg per day) stimulated an increase in the tibial epiphyseal width (Fig. 1a) to 139%, the

higher dose (103 µg per day) to 168% compared with controls. The effect of IGF I was similar to that obtained in hypophysectomized rats treated with human growth hormone (HGH); whether administered by continuous s.c. infusion or by two intraperitoneal injections daily, HGH (12.5 or 25 mU per day) caused stimulation of growth to 157 and 176%, respectively,

Figure 1b shows DNA synthesis in vitro in costal cartilages of the same rats. IGF I (43 µg per day) increased ³H-TdR incorporation to 171%, and 103 µg per day to 239% compared with controls. Again the effect of HGH was similar: an increase to 172% (12.5 mU per day) and 265% (25 mU per day) was observed. The gain in body weight (Fig. 1c) during IGF I infusion was also comparable to that obtained during HGH administration.

Thus, IGF I administered to hypophysectomized rats mimics the effects of GH on these important indices of growth. Longitudinal growth is stimulated directly by IGF I. Our results strongly support the somatomedin concept2; however, they do not exclude other indirect and direct effects of GH on various tissues.

We thank Mr Meier and Dr Maier for supplying the hypophysectomized rats and Cristina Hauri and Therese Steiner for technical assistance. This work was supported by grant no. 3.380-0.78 from the Swiss NSF.

Received 12 November 1981, accepted 2 February 1982

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³H-noradrenaline release potentiated in a clonal nerve cell line by low-intensity pulsed magnetic fields

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Recent studies have shown that extremely low-frequency (ELF) electric fields¹ and radiofrequency amplitude-modulated electromagnetic fields^{2,3,28} can influence ion fluxes in neural tissue. Furthermore, the application of low-frequency magnetic (LFM) fields in the successful treatment of non-union fractures and pseudo-arthrosis has been widely reported4-6. Stimulated by these findings and the wide variety of effects reported in other biological systems⁷⁻¹⁰, we have set out to establish a model system for studying the biological effects of such low-frequency fields that readily allows analysis of their mechanism of action. Clonal lines in tissue culture are an obvious choice for such a model, first, because the analysis is simplified by the presence of only one cell type and second, because the geometry of the biological sample can be specified to allow precise quantitation of applied field strengths and induced current densities. One such clonal line, designated PC12, is a likely candidate for such an approach as it expresses many properties of differentiated sympathetic neurones¹¹⁻¹⁶, including calcium-dependent release of neurotransmitters^{11,12,14}. We report here that ³H-noradrenaline(NA) release from PC12 cells is stimulated by an inductively coupled 500-Hz LFM field with a magnitude comparable with certain cholinergic stimuli in this system.

PC12 cells are derived from a transplantable rat phaeochromocytoma¹⁵; they synthesize, store and release dopamine, noradrenaline and acetylcholine¹¹⁻¹³ and express sodium action potentials¹⁶. In these experiments, PC12 cells were grown in Dulbecco's modified Eagle's medium containing 5 g l⁻¹ glucose, supplemented with 10% calf serum and 5% horse serum in the absence of nerve growth factor.

A magnetic field was generated by passing current through a pair of concentric 400-turn coils, 10.6 cm in diameter, separated by 14.7 cm. Two culture dishes were placed symmetrically between the coils at heights of 4.95 and 9.75 cm from the bottom coil. The changing magnetic field induces circulating eddy currents in the conducting medium in the dishes, with the current density increasing from zero at the centre to a maximum at a distance approximately equal to the coil radius. The range of currents so induced was limited by containing the cells in an annulus. This was achieved by gluing a small inverted Petri dish into the centre of the 60-mm sample dish and plating the cells in the annular channel so formed. This limits the range of induced current densities to within ±20% of the mean¹⁷. The magnetic field was generated between the coils by wiring them in parallel to a pulse generator and driving circuit similar to that used in clinical trials for the treatment of non-union fractures in Newcastle and London (P. O. Byrne and D. Outram, in preparation). The generator gives a train of square pulses with a range of different pulse durations, frequencies and intensities17

For these experiments we used a pulse width of 0.6 ms, with 1.4 ms between each pulse, giving an overall frequency of 500 Hz. The current waveform was smoothed due to the large inductance of the coils (Fig. 1). The applied magnetic field will have the same form as the current in the coils and can be calculated from the geometry of the coils and the current 17,18 . The maximum field applied to the sample was 8.5×10^{-4} WB m $^{-2}$ (8.5 G) and the minimum 1.6×10^{-4} Wb m $^{-2}$ (1.6 G). The rate of change of the magnetic field, calculated from the voltage induced in a search coil, varied between +1.8 and -0.9 Wb m $^{-2}$ s $^{-1}$, and the calculated circulating eddy current density varied between +0.025 and -0.013 A m $^{-2}$, inducing an electric field ranging from +3.8 $\times10^{-2}$ to -1.9×10^{-2} V m $^{-1}$.

³H-NA release was measured using logarithmic-phase PC12 cells by a modification of the procedure of Greene and Rein¹². After 2 h incubation with ³H-NA, cytoplasmic NA stores were

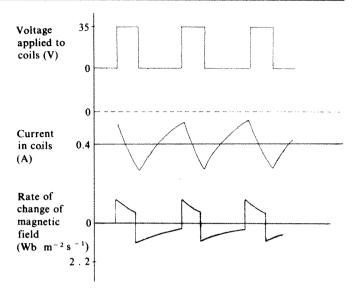


Fig. 1 Waveform generated by the low-frequency magnetic field generator. The magnetic field was calculated from the total measured current I in the coils using the formula

$$B_{z} = \mu \mu \frac{I}{2} \times \frac{na^{2}}{2} \left[\frac{1}{(z_{1}^{2} + a^{2})^{3/2}} + \frac{1}{(z_{2}^{2} + a^{2})^{3/2}} \right]$$

where a = radius of the coils, n = number of turns, and z_1 and z_2 are distances from each coil to point of calculation. $\partial B_z/\partial t$ was calculated from the voltage (V) induced in a search coil of area A and N turns: $V = NA \partial B/\partial t$ (refs 17, 18).

largely depleted as the NA was spontaneously released from the cells during a 3.5-h washout period. Release was then monitored for 12 sequential 15-min periods. At the 105- and 120-min time periods (designated experimental), two dishes were removed from the Numetal shield, which protects the cells from stray magnetic fields, and placed between the coils for a total of 13 min. To eliminate the possibility that LFM fields dislodge cells into the medium, thereby generating artificially high values, medium from the cells was centrifuged at 2,200g for 5 min before assaying for radioactivity. The pellets were solubilized and counted separately.

In all 10 trials in which the LFM fields were applied, release values (c.p.m.) obtained at 105 and 120 min were always higher than the values obtained from the two preceding time periods. To estimate the extent of the observed stimulation, the first six release values for all 22 trials were fitted to the polynomial function $\log Y = \phi(x) + N + T$ from which the control curve of Fig. 2 is obtained; Y represents the c.p.m. in the supernatants, x is the time in minutes, N is a normalizing term allowing direct comparison between dishes and T is a term inserted for the experimental period¹⁹. In the control dishes (n = 12) the observed values for the experimental periods fitted within 1 s.d. of those obtained by extrapolating the polynomial function,

Table 1 ³ H-noradrenaline release from PC12 cells							
c.p.m. expressed as %	Time (min)						
of cell content	n	90	105	120	135		
Control	12	0.717 ± 0.039	0.686 ± 0.046	0.657 ± 0.044	0.628 ± 0.033		
Experimental	10	0.717 ± 0.039	0.875 ± 0.064	0.838 ± 0.061	0.628 ± 0.033		
Experimental + 15 mM Mg ²⁺	8	0.717 ± 0.039	0.673 ± 0.015	0.686 ± 0.02	0.628 ± 0.033		
Pellets	10	0.049 ± 0.012	0.05 ± 0.014	0.045 ± 0.013	0.06 ± 0.024		

Release values were generated by regression analysis and expressed graphically as in Fig. 2. In the elevated Mg²⁺ trials the medium for the 105- and 120-min time periods, during field stimulation, contained 15 mM MgSO₄. The aspirated medium was treated as described in Fig. 2 legend. Pellets obtained from spinning aspirated medium (experimental series) at 2,200g for 5 min were solubilized in 1.5 ml of 1 M NaOH and assayed for radioactivity in 10 ml Instagel. All values are expressed as a percentage of the residual intracellular radioactivity and are shown as mean ± s.e.m.

showing that the washout curve varies smoothly with time. The experimental values (during LFM field stimulation) were elevated above the controls, with the treatment effect being highly significant (P < 0.001).

Expressing the stimulated release as the difference between the control and experimental values relative to the control value for a given time period, a percentage stimulation (±s.e.m.) of $27.5\% \pm 4.9$ is obtained for both experimental time periods. This elevation is likely to be due to increased release of NA from the cells in monolayer and not to contamination by LFM field-induced floating cells, because the pellet counts for the experimental periods are not significantly different (P > 0.5)from those before or after it (see Table 1). The temperature of the coils was continuously monitored and did not rise above the 37 °C of the CO₂ incubator.

In eight further trials in which 15 mM Mg²⁺ was included in the medium for the experimental periods, the rate of release in control washout curves did not alter, but the stimulated release was abolished (see Table 1). The stimulated release

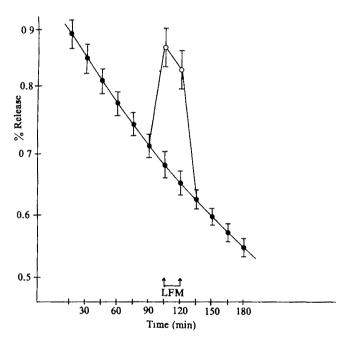


Fig. 2 Effect of a 500-Hz pulsed magnetic field on noradrenaline release from PC12 cells. This curve was generated by regression analysis from a population of 22 curves. Cells were grown in collagen-coated annular dishes made by gluing an inverted 35-mm dish to a 60-mm dish. All manipulations were carried out in a 37 °C warm room. Logarithmic cells (~1×10⁶ cells per dish) were incubated for 2 h in Dulbecco's modified Eagle's medium containing 1 mM (-)sodium; ascorbate and 2 μCi ³H-(-) NA(11 Ci/mmot⁻¹; Amersham) in a CO₂ (7%) incubator maintained at 37 °C. The radioactive medium was then removed and the cells washed three times with fresh medium (at 37 °C). The cells were incubated in a 37 °C/CO₂ atmosphere for an additional 3.5 h in 1.5 ml fresh medium. During this time, the medium was changed after 2 h and then at 30-min intervals. Thereafter the medium (1.5 ml) was changed every 15 min for 12 consecutive periods. The medium from these changes was transferred to polypropylene tubes and centrifuged at 2,200g for 5 min to pellet gently any floating cells. The supernatants were then assayed for radioactivity in 10 ml Instagel (Packard). At the end of each experiment, radioactivity remaining in the cells was assayed by scraping the cells sequentially in 0.5 ml 1 M HCl, 0.5 ml 1 M NaOH and 0.5 ml water. The pooled scrapings were then assayed for radioactivity as above. Release values were calculated from the ratio of the c.p.m. in the supernatant, at a given time period, to the total residual intracellular radioactivity at the end of the experiment. The mean total intracellular count (±s.e.m.) was $389,018\pm26,827$. We have shown previously that 84% of the radioactivity released from the cells is unmetabolized NA¹². \bullet , Control curve, O, Stimulated points.

values in the presence of elevated Mg^{2+} were not significantly different from the controls (P>0.3). In three of the trials, following exposure to 51.5 mM K⁺ (ref. 11) following the period of stimulation by the LFM, the cells released a substantial proportion of their NA store, indicating that irreversible damage had not occurred.

These results suggest that PC12 cells can be used as a model system for studying the effects of LFM fields on neuronal functions. Release of catecholamines (spontaneous or preloaded with ³H-NA) from PC12 cells can be induced by depolarizing concentrations of K⁺ (refs 11, 14), the sodium ionophore veratridine 11, or by activating acetylcholine receptors with nicotine¹² or carbamylcholine¹⁴. Whereas the maximum stimulation of NA release induced by nicotine $(10 \,\mu\text{M})^{12}$ is ~300% in similar experimental conditions to those used here, the acetylcholine analogue carbamylcholine (100 µM) induced a 60% stimulation of release (L. A. Greene and G.R., unpublished observation). The 27.5% stimulation observed here may not be maximal, as a dose-response curve has not yet been established, but is still of comparable magnitude with that obtained with certain cholinergic stimuli.

Previous studies on Ca2+ efflux from chick and cat cerebrum have stressed the importance of a 6-20-Hz frequency 'win $dow^{20,21}$, although an initial report on efflux of γ -aminobutyric acid in the cat cortex showed a small increase induced by a 200-Hz directly coupled signal²². In the experiments reported here release was induced by a 500-Hz pulsatile waveform. Clearly the frequency dependence needs further investigation and studies are under way to determine the effect of different stimulation frequencies and the importance of the pulse shape and modulation.

The observation that Mg2+ inhibits the LFM field-induced release from PC12 cells indicates a requirement for extracellular Ca²⁺, as Mg²⁺ has been shown to be a specific Ca²⁺ antagonist in several biological systems²³. Because depolarization-induced release is also inhibited by Mg²⁺ in PC12 cells^{11,12,14}, it is possible that LFM field-induced release occurs via a similar exocytotic mechanism in which Ca2+ fluxes are involved24. However, on biophysical grounds alone, the direct imposition of Ca2+ entry by this LFM field is an unlikely hypothesis, because we estimate that the maximum electric field intensity is $\sim 4 \times 10^{-2} \text{ V m}^{-1}$, far less than the 10^3 V m^{-1} field associated with a typical action potential. A more plausible hypothesis is that the cation binding sites on the outer surface of the plasma membrane may be highly sensitive to such weak stimuli²⁰ and that subsequent cooperative changes in Ca2+ binding may influence membrane stability, thereby promoting Ca²⁺ entry and vesicular release.

Clearly, this physical stimulus offers a new approach for studying the mechanisms involved in stimulus-secretion coupling²⁴ as well as having important and widespread clinical implications.

We thank Dr Michael Lunt for the design and characterization of the physical approach, Mr Nigel Hathway for assistance in analysing the results and the Smith's Charity for supporting R.D. during this study.

Received 16 September 1981; accepted 26 January 1982

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Agonist selectivity and second messenger concentration in Ca²⁺-mediated secretion

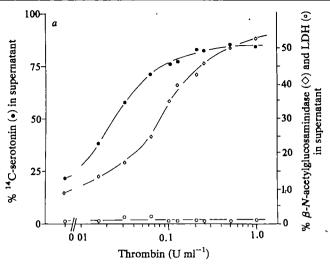
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Cells typically carry receptors for several excitatory and/or inhibitory agonists. In many cases the pattern of response observed is characteristic of the particular agonist used 1-4. These differences in the nature of the responses cannot always be explained on the basis that the agonists use different intracellular second messengers³⁻⁵. For example, in the blood platelet, Ca2+ seems to be the second messenger6 for such excitatory agonists as ADP, adrenaline, thrombin, collagen and 11-epoxymethanoprostaglandin H₂ (U-46619). These agonists cause secretion of serotonin, ADP and ATP from the amine storage granules whereas secretion of acid hydrolases from the lysosomes has been reported to occur only on stimulation by higher concentrations of collagen and thrombin⁷⁻¹¹. We have investigated this agonist selectivity and report here results suggesting that it is not related to cytosolic Ca²⁺ concentration.

Figure 1a illustrates the dose-response curves observed for release of serotonin, β -N-acetylglucosaminidase and lactate dehydrogenase when washed human platelets are challenged with thrombin. The concentration (EC₅₀) of thrombin required for half-maximal release of serotonin (0.02 U ml⁻¹) is significantly lower than that of β -N-acetylglucosaminidase (0.09 U ml⁻¹), while no significant release of the cytosolic marker enzyme, lactate dehydrogenase, is observed over the range of thrombin concentration tested. In accord with these observations, data from experiments performed in different conditions reveal a nonlinear relationship between the thrombin-induced release of serotonin and β-N-acetylglucosaminidase from intact platelets, with serotonin being preferentially released at low thrombin concentration (Fig. 1b). This selectivity in the response to thrombin could be explained if secretion of acid hydrolases occurs only when the cytosolic Ca²⁺ concentration is higher than is required for secretion from the amine storage granules. We have therefore examined secretion of serotonin, ADP and β -N-acetylglucosaminidase by washed platelets rendered permeable by exposure to an intense electric field, thereby allowing their internal calcium concentration to be varied1

shows the release of serotonin, β -Nacetylglucosaminidase and lactate dehydrogenase measured as a function of Ca²⁺ concentration for platelets rendered permeable and then challenged with Ca2+ buffers. Release of β -N-acetylglucosaminidase is observed over the same range of Ca2+ concentrations as release of serotonin, but in accord with earlier observations¹² there is no significant Ca²⁺-dependent release of lactate dehydrogenase. The pattern of serotonin and B-N-acetylglucosaminidase release observed in the permeable cells (Fig. 2) contrasts markedly with that observed when the intact cells are challenged with thrombin (Fig. 1a). From the log₁₀ dose-response relationship observed in several experiments using the permeable cells, the EC₅₀ for Ca²⁺-induced release of β -N-acetylglucosaminidase is calculated to be 1.8 \pm



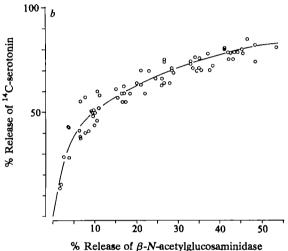


Fig. 1 a, The relationship between the extents of release of -C-serotonin (\bullet), β -N-acetylglucosaminidase (\diamond) and lactate dehydrogenase (LDH, O) and thrombin concentration for intact human platelets. Human blood platelets were suspended in 0.28 M sucrose containing 0.02 M PIPES, 0.01 M K+-glutamate, 2 mM MgCl₂ and 0.4 mM EGTA pH 6.6 as described previously Aliquots (0.25 ml) were challenged with various concentrations of thrombin for 5 min at 20 °C before being centrifuged for 2 min at 8,000g. The supernatant fraction was removed and aliquots taken for assay of ¹⁴C content (50 µl), lactate dehydrogenase (100 μ l) and β -N-acetylglucosaminidase (50 μ l). Lactate dehydrogenase was assayed spectrophotometrically and β -N-acetylglucosaminidase fluorimetrically The C, lactate dehydrogenase was assayed spectrophotometrically and β -N-acetylglucosaminidase fluorimetrically S. The C, lactate dehydrogenase was assayed spectrophotometrically and β -N-acetylglucosaminidase fluorimetrically S. The C, lactate dehydrogenase was assayed spectrophotometrically S. The C, lactate dehydrogenase was assayed spectrophotometrically S. The C, lactate dehydrogenase was assayed spectrophotometrically S. The S. The S. The C. Lactate dehydrogenase was assayed spectrophotometrically S. The S. drogenase and β-N-acetylglucosaminidase contents of the supernatant fractions are expressed as per cent of the total content of the platelet suspension. The total contents of lactate dehydrogenase and B-N-acetylglucosaminidase in the platelet suspension were typically 120 mU ml⁻¹ and 10.0 mU ml⁻¹ respectively. Essentially similar results were obtained when the platelets were suspended in media at pH 7.4 and over a range of Ca-EGTA buffers (10 mM) corresponding to $\sim 10^{-8}-10^{-5}$ M Ca²⁺. b, The relative amounts of β -N-acetylglucosaminidase compared with ¹⁴C-serotonin released from intact platelets incubated in various concentrations of thrombin and in conditions as in a. The amounts released are expressed as a per cent of their cellular content.

 $0.1 \,\mu\text{M}$ (n = 7), in good agreement with the value of $1.9 \pm$ 0.9 µM determined previously for serotonin release 12. The EC₅₀ is independent of the concentration of EGTA in the Ca²⁺ buffer over the range 5-50 mM, suggesting that this release results from the direct action of micromolar concentrations of Ca2+ on the secretory apparatus, rather than through Ca²⁺-induced release of Ca^{2+} from intracellular stores. The release of β -Nacetylglucosaminidase in this system is smaller than that observed for serotonin, a finding which agrees with the relative

release of B-N-acetylglucosaminidase and serotonin observed both here (Fig. 1) and in previous studies^{7,9} on stimulation of intact platelets by high concentrations of thrombin.

Release of β -N-acetyglucosaminidase is complete within seconds of challenging permeable cells with $10^{-5}\,\mathrm{M\,Ca^{2+}}$, and in a variety of conditions is closely correlated (Fig. 3) with release of both serotonin and ADP, the major nucleotide present in the amine storage granules of human platelets¹³. In contrast, no correlation is observed in these conditions between the release of these constituents and that of lactate dehydrogenase (Fig. 3). In the case of ATP an intermediate situation is observed, as might be expected because significant concentrations of this nucleotide are present in both the cytosol and the amine storage granules of human platelets¹³.

Our data therefore provide further evidence that exocytosis can be induced in human platelets as an immediate consequence of an increase in cytosolic Ca2+ concentration and demonstrate

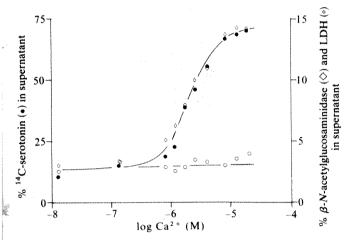


Fig. 2 The relationship between the extents of release of ¹⁴Cserotonin (•), β-N-acetylglucosaminidase (◊) and lactate dehydrogenase (O) and the Ca2+ concentration in human platelets rendered permeable by exposure to a 20-kV electric field. Human blood platelets were obtained and suspended in 0.28 M sucrose containing 0.2 M PIPES, 0.01 M K+-glutamate, 5 mM ATP, 7 mM MgCl₂ and 0.1 mM acetylsalicylic acid pH 6.6 as described previously ¹². After addition of 0.4 mM EGTA the suspension was exposed to an electric field of 20,000 V cm⁻¹ with a time constant of 30 μ s as described previously ¹² and aliquots (0.25 ml) were then immediately challenged with 10 mM Ca-EGTA buffers. The free ionized calcium concentrations were calculated using equilibrium constants of $10^{-5.928}\,\mathrm{M}$ (Ca-EGTA), $10^{-1.445}\,\mathrm{M}$ (Mg-EGTA), $10^{-3.538}\,\mathrm{M}$ (Ca-ATP) and $10^{-3.675}\,\mathrm{M}$ (Mg-ATP)¹⁶. After incubation for 5 min at 22 °C the samples were centrifuged for 2 min at 8,000g and the supernatant fractions removed. Aliquots were assayed for 14 C-serotonin, lactate dehydrogenase and β -Nacetylglucosaminidase as described in Fig. 1. The contents are expressed as a per cent of the total contents of the platelet suspension. Before exposure to the electric field, 5% of the 14 and 2% of the lactate dehydrogenase and the β -N-acetylglucosaminidase were present in the extracellular medium. Control studies have shown that: (1) the properties of release of 14 C-serotonin and β -N-acetylglucosaminidase do not differ significantly if acetylsalicylic acid is omitted from the suspending buffer; (2) leakage of ATP, which occurs in the presence of 10⁻⁹ M ⁺, is complete at the earliest time (30 s) that could be examined after exposure to the electric field; and (3) no significant leakage C-serotonin, lactate dehydrogenase, B-N-acetylglucosaminidase or enolase resulted from exposure to the electric field in the presence of $10^{-9}\,\mathrm{M\,Ca}^{2+}$. These latter observations establish that the membranes of the amine storage granules and the lysosomes are not damaged by exposure to the electric field and that the 'pores' created in the plasma membrane are large enough to allow free passage of molecules of molecular weight <500 but too small to permit leakage of proteins of molecular weight >80,000. Further studies are required to define the behaviour in this system of cellular constituents having molecular weights between these two values.

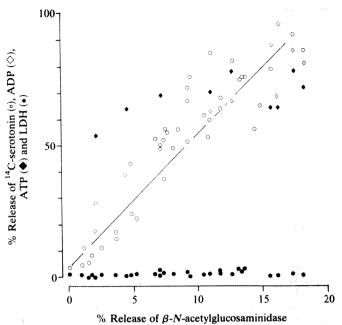


Fig. 3 The relative amounts of β -N-acetylglucosaminidase with -serotonin (○), ADP (♦), ATP (♦) and lactate dehydrogenase () released from human platelets incubated in various conditions after exposure to an electric field as described for Fig. 2. Human platelets were prepared, washed and rendered permeable as described for Fig. 2 except that where ADP and ATP were measured, the medium contained 2 mM MgCl₂ and ATP was omitted. ATP and ADP were assayed as described previously The amounts of all components assayed are expressed as per cent of total cellular content. The total contents of ATP and ADP in the platelet suspension were 3.3 and 2.2 μ M respectively. After exposure to the electric field in the presence of 10^{-9} M Ca²⁺ the ATP and ADP contents of the supernatant fraction were 2.5 and $0.4 \mu M$ respectively. The correlation coefficients between β -N-acetylglucosaminidase and 14 C-serotonin, ADP, ATP and lactate dehydrogenase are 0.92 (n=40), 0.95 (n=9), 0.60 (n=9) and 0.37 (n = 33) respectively. The line shown is a linear regression of 14 C-serotonin release on β -N-acetylglucosaminidase release and has a slope of 5.0 ± 0.4 ($\pm s.d.$).

that the increase in Ca2+ concentration required is not significantly different for platelet amine storage granule and lysosomal secretion. Hence, the agonist selectivity observed for secretion of the constituents of these two granules is likely to result from an event in the stimulus-response coupling mechanism other than that responsible for altering the concentration of Ca² in the platelet cytosol.

Received 7 September 1981; accepted 5 February 1982.

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Monoclonal antibody against human IFN-γ

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Monoclonal antibodies have proved extremely useful for the unambiguous identification, quantification and large-scale purification of a given antigen or a specific epitope. As they are highly specific for a given antigenic determinant, these antibodies are superior to conventional polyclonal antibodies. Since the discovery1 that mouse spleen B lymphocytes can be fused with mouse B-myeloma cells to yield hybrid cells (hybridomas) secreting specific antibodies derived from the spleen cell fusion partner, several modifications of this method have been used to improve the yield of surviving antibodysecreting hybridomas. We have now included, 5 days after the fusion proceedure, an additional step which stabilizes the hybrids against gene loss and improves growth conditions. We report here that, using this method, we have established several stable mouse hybridoma lines secreting monoclonal antibodies to human γ-interferon (HuIFN-γ).

HuIFN- γ was obtained from buffy coats after induction with concanavalin A and subsequently purified by controlled-pore glass adsorption and Sephacryl S200 column chromatography². BALB/c mice were immunized intraperitoneally (i.p.) every third week with 20 units of the HuIFN- γ in a mixture of incomplete Freund's adjuvant and N-acetylmuramyl-L-alanyl D-isoglutamine. Three days before fusion the mice were given a final booster (seventh immunization) subcutaneously of 60,000 units of HuIFN- γ in incomplete Freund's adjuvant. The fusion of mouse spleen lymphocytes and mouse (FO) myeloma cells was performed as described by Hochkeppel *et al.*³. After fusion, the hybridomas were first cultured for 5 days in hypoxanthine-aminopterin-thymidine (HAT) medium containing

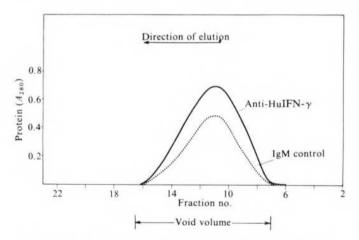


Fig. 1 Elution profile of monoclonal anti-HuIFN- γ on Sephadex G-200. Hybridoma supernatants (10 ml) containing \sim 1–5 μg ml $^{-1}$ of antibody were precipitated with 40% ammonium sulphate (ice-cold) and the precipitated proteins, after resuspension in 200 μl Tris-buffered saline pH 7.4, were further purified by filtration on a 10 ml Sephadex G-200 filtration column in Tris-buffered saline, pH 7.4. At this protein concentration no IgG protein peak was visible. The antibody eluted with the void volume and had an elution profile identical to that of an IgM control.

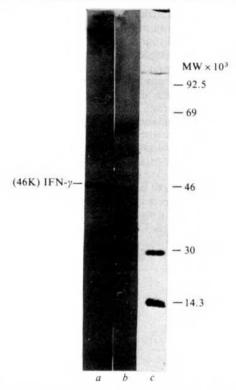


Fig. 2 ELISA using HuIFN-y and monoclonal anti-HuIFN-y. Partially purified HuIFN-y which had been pretreated with 0.1 M SDS alone was separated, in parallel with ¹⁴C-labelled standard proteins, by SDS-polyacrylamide gel electrophoresis according to Laemmli⁵. All proteins were electrophoretically transferred to a nitrocellulose sheet according to Towbin *et al.*⁶. While the section of the blot containing marker proteins was subjected to autoradiography, ELISA was performed on the section containing HuIFN-γ. First the blot was saturated for 2 h with 3% bovine serum albumin (BSA)/5% horse serum to block all the nonspecific binding sites. After washing with Tris-buffered saline, monoclonal anti-HuIFN-γ (10 μg ml-1) purified from hybridoma culture supernatants was added at a dilution of 1:50 and incubated at 25 °C for 16 h. Subsequently rabbit IgG directed against mouse IgM was added at a 1:200 dilution in 5% horse serum, 0.5% BSA (4 h, 25 °C) and finally goat peroxidase-conjugated IgG directed against rabbit IgG (1:500; 2 h at 25 °C). The reaction was followed by observing the colour change with O-dianisidine used as a substrate for H2O2. a, HuIFN-y plus monoclonal anti-HuIFN-γ; b, HuIFN-γ plus medium (control); c, 14C-labelled standard proteins.

Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% fetal calf serum (FCS) and 1% methylcellulose. After 1 week, with 20% of hybridomas surviving, half of each individual culture was injected into the peritoneal cavity of separate BALB/c mice which had been pretreated with pristane for 1 week⁴. (The other half was cultured further in HAT medium.) One week after injection, ascites fluids were collected and the hybrids cultured in IMDM +10% FCS. Of 16 stable lines secreting monoclonal anti-HuIFN- γ , 15 were isolated from those cultures which had been passaged through a mouse after fusion.

These hybridomas were subcloned in Greiner 'single clone' 24-well tissue culture plates in which each of the wells was divided into 16 subsquares. As the FO hybridomas grow attached to the well surface, these subdivided wells are a convenient tool for subcloning. Growing colonies were tested for specific anti-HuIFN- γ secretion in an antiviral neutralization test on human CCL 23 cells. Briefly, the surface of microtitre immunoassay plates were coated with rabbit anti-mouse immunoglobulin, and the supernatants of individual mouse hybridoma cultures were incubated in these plates for 16 h. After a final wash, 10 units of HuIFN- γ were added to each

individual well and the plates were incubated at 37 °C for 4 h. The remaining antiviral activity in each cup was then titrated in a viral RNA reduction assay using vesicular stomatitis virus on human CCL 23 cells. By thus testing the individual anti-IFN-γ hybridoma lines for constant antibody secretion over a period of 6 months, 16 stable mouse hybridoma lines secreting monoclonal anti-HuIFN-y were established. So far, we have characterized the monoclonal antibody of one of these lines.

To identify the immunoglobulin species of this monoclonal anti-HuIFN-y, the antibody was purified from hybridoma supernatants by ammonium sulphate (40%) precipitation followed by gel filtration on Sephadex G-200. The anti-HuIFN-γ eluted in the void volume with an elution profile identical to that of an IgM control (Fig. 1). The specificity of the purified monoclonal anti-HuIFN-y was tested further in an enzyme-linked immunosorbent assay (ELISA) against HuIFNy which had been separated by SDS-polyacrylamide gel electrophoresis⁵ (omitting β -mercaptoethanol treatment of the HuIFN-y sample) and electrophoretically transferred to a nitrocellulose sheet according to Towbin et al.6. Figure 2 shows

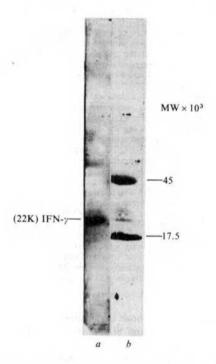


Fig. 3 ELISA using HuIFN-y and monoclonal anti-HuIFN-y. Partially purified HuIFN-y which had been pretreated with SDS and β -mercaptoethanol was separated in parallel with standard proteins by SDS-polyacrylamide gel electrophoresis according to Laemmli⁵. After electrophoretic transfer of all proteins to a nitrocellulose sheet, the section containing the standard proteins was stained with amido black. An ELISA was performed on the section containing the HuIFN-y as described in Fig. 2 legend. a, HuIFN-y plus monoclonal anti-HuIFN-y; b, standard proteins stained with amido black.

that the monoclonal anti-HuIFN-y specifically reacted with the HuIFN-y preparation at a position corresponding to molecular weight (MW) 46,000 (46K). The same experiment was carried out after treatment of the IFN-y preparation before SDSpolyacrylamide gel electrophoresis with SDS and β -mercaptoethanol to avoid possible dimerization of the HuIFN-y molecule. In these conditions the HuIFN-y blotted on to nitrocellulose reacted with monclonal anti-HuIFN-y at a position corresponding to MW 22,000 (Fig. 3). These results suggest that monoclonal anti-HuIFN-y is specifically directed either against two different HuIFN-y subspecies or against the dimer in one case (Fig. 2) and against the monomer in another (Fig. 3). However, the shifting of the immuno-reacting HuIFN-y

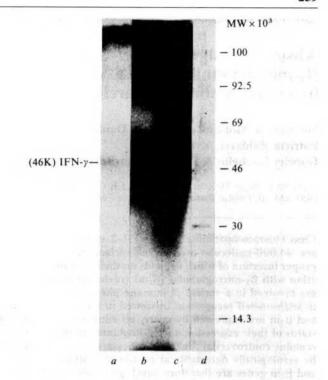


Fig. 4 Autoradiograph of a 15% SDS-polyacrylamide gel⁵ containing: *a*, immuno-purified ¹²⁵I-HuIFN-γ; *b*, wash fraction; *c*, original ¹²⁵I-HuIFN-γ; and *d*, ¹⁴C-labelled protein markers. Partially purified 125 I-IFN-y which had been incubated first with monoclonal mouse anti-HuIFN-y for 16 h and then with rabbit IgG antibody directed against mouse IgM (16 h) was precipitated with S. aureus protein A. The precipitate was washed four times with Tris-buffered saline, then the specifically bound ¹²⁵I-IFN-γ (pretreated with SDS, but not β -mercaptoethanol) was separated on an SDS-polyacrylamide gel⁵ in parallel with 14 C-protein markers, the wash fraction and the original 125 I-HuIFN- γ sample.

band after β -mercaptoethanol treatment makes the latter possibility more likely.

In a further experiment, partially purified HuIFN-y which had been labelled with 125I according to Hunter et al.7 was incubated first with monoclonal mouse anti-HuIFN-y and subsequently with a rabbit (IgG) anti-mouse IgM antibody. This complex was then precipitated with IgG-specific Staphylococcus aureus protein A. After several washes of the precipitate, the specifically bound ¹²⁵I-labelled IFN-γ was separated on an SDS-polyacrylamide gel according to Laemmli⁵ (without \(\beta\)mercaptoethanol treatment) in parallel with 14C-labelled protein markers. Autoradiography of the dried gel (Fig. 4) showed that monoclonal anti-HuIFN-y bound to S. aureus protein A, specifically precipitated ¹²⁵I-IFN-γ at 46,000 MW. Finally in an ELISA there was no cross-reaction of anti-HuIFN-y with either HuIFN-β or HuIFN-α which had been electrophoretically transferred from an SDS-polyacrylamide gel on to a nitrocellulose sheet, nor did monoclonal anti-HuIFN-β crossreact with HuIFN-y (data not shown).

HuIFN-β was a gift from Rentschler/Laupheim Co. and HuIFN-α was given by H. Mohr. M.L. is Bevoegdverklaard Navorser of the Belgian National Fonds voor Wetenschappelyk Onderzoek.

Received 10 November 1981; accepted 21 January 1982.

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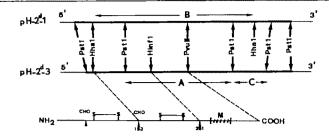
Absence of significant H-2 and β_2 -microglobulin mRNA expression by mouse embryonal carcinoma cells

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Class I histocompatibility antigens (H-2 antigens in the mouse) are 44,000-molecular weight cell-surface glycoproteins, the proper insertion of which depends on their noncovalent association with β_2 -microglobulin (β_2 m) (reviewed in ref. 1). They are involved in a variety of immune phenomena, particularly in self/non-self recognition (reviewed in ref. 2). H-2 antigens and B2m are expressed by nearly all adult somatic cells. The status of their expression on pre-implantation mouse embryos remains controversial^{3,4} although they are generally agreed to be serologically detectable at post-implantation stages⁵. H-2 and B₂m genes are therefore developmentally regulated. H-2 antigens have not been found on murine embryonal carcinoma (EC) cells⁶, which are widely used as an alternative to studying normal early embryonic cells⁷⁻⁹, but they become detectable during in vitro differentiation of EC cells¹⁰⁻¹². During studies aimed at understanding the genetic mechanisms involved in the expression of H-2 antigens and β_2 m during differentiation, cDNAs reverse transcribed from mRNAs coding for H-2 or H-2-related heavy chains and β2m have been characterized¹³⁻¹⁶. H-2 and β₂m cDNAs were used as probes to screen total poly(A)+ RNAs from various cell types. We report here that trace amounts of poly(A)+ RNA from EC cells were found to hybridize with these probes, finding which contrasts with results obtained on poly(A)+ RNA prepared from differentiated cells. These results, taken together with the absence of major rearrangements in H-2 and β2m genes during differentiation, suggest that H-2 and β₂m expression is likely to be controlled at the level of transcription.

Figure 1 legend describes the probes used in the study. Poly(A)+ RNAs were extracted from liver, spleen and kidney of DBA/2 (H-2^d) and 129/Sv (H-2^{bc}) mice. (These animals are respectively syngeneic to SL2 tumour cells, from which the to most of the EC lines used in the present study¹⁷). The poly(A)⁺ RNAs were electrophoraged in account study¹⁷). red to nitrocellulose sheets and finally hybridized with ³²P-labelled probes ¹⁸. Probes H-2A, B and C revealed the same unique band, corresponding to RNAs of ~1,800 nucleotides (Fig. 2, lanes b, c, results shown for H-2B probe), a size in good agreement with that estimated (17S) from sedimentation through sucrose gradient¹⁹. In the various H-2 cDNA clones studied so far^{20,21}, the 3'-untranslated region is ~480 nucleotides long, excluding the poly(A) tail. The coding region, estimated from the known amino acid sequence of H-2K^b (ref. 22), must be about 1,000 nucleotides long; assuming the poly(A) tail does not exceed 100 nucleotides, the length of the 5'untranslated region in H-2 mRNAs should be 100-300 nucleotides. We have detected no size difference nor any heterogeneity in the two mouse strains or in the various tissues tested (Fig. 2, lanes b,c and data not shown). The absence of heterogeneity implies that members of the H-2 multigene family, when expressed, yield mRNAs of an approximately similar size (in this size range, we could only detect a difference >100 nucleotides). Finally, if non-H-2 mRNAs are expressed and carry the reiterated sequence detected by probe C, they must fall within the same size ranges as H-2 mRNAs. Hybridization with the



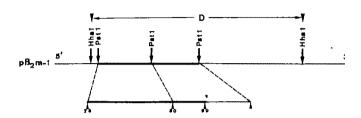


Fig. 1 Top. The restriction maps of the cDNA inserts of the recombinant plasmids $pH-2^d-3$ and $pH-2^d-1$ (refs 20, 24) are presented in correspondence with the linear structure of an H-2 antigen molecule M indicates the membrane spanning region with the external part of the molecule on the NH2-terminal side and the cytoplasmic fragment on the COOHterminal side. A, B and C represent restriction fragments used as radiolabelled probes in experiments described in the text. Probe H-2A is the 578-base pair (bp) Psh-Psh of pH-2^d-3²⁰ From its 5' end, it extends over the From its 5' end, it extends over the COOH-terminal part of an H-2 molecule (nucleotides 180-498, PvuII site), corresponding to part of the third external domain, transmembrane and cytoplasmic regions; the probe extends downstream over the first 260 bp of the noncoding region. This fragment does not contain a region hybridizing with a sequence highly repeated in the genome²⁴. Probe H-2B is the 938-bp Hha-Hha fragment of pH-2⁴-1¹³. The 5' part (up to the PvuII site) encodes for a nearly complete third extracellular domain, transmembrane and cyto-The 327-bp noncoding region downstream contains at its 3' end a stretch of 20 nucleotides belonging to the Alu type II sequence Probe H-2C is the 160-bp PstI-PstI fragment of pH-2d-3. Its 3' end includes the Alu type II sequence. This sequence is repeated a minimum of 40,000 times in the haploid genome of the mouse^{14,24}. Bottom The restriction map of the cDNA insert of the recombinant plasmid $p\beta_2$ m-1 is presented in correspondence with the linear structure of the β_2 m molecule. This clone has been obtained by screening a mouse cDNA library with a recently cloned human β_2 m cDNA probe¹⁶. p β 2m-1 includes most of the sequence coding for β_2 m and about 100 additional basepairs of the 3'-untranslated region. The restriction fragment D was used as the β_2 m probe.

 β_2 m probe reveals two bands of similar intensities, migrating approximately as 1,000 and 800 nucleotides in both DBA/2 (Fig. 2, lane d, and Fig. 3A, lanes e, f) and 129/Sv (Fig. 3A lane g) poly(A)⁺ RNAs. These two bands could correspond to either the same coding sequence associated with different untranslated regions or to products of two different genes.

Poly(A)+ RNAs were extracted from several EC lines, F9 PCC3 and PCC4-aza¹² and analysed as above. Using probes prepared at the usual specific radioactivity (2-3×108 c.p.m. pe μg), no poly(A)+ RNA was found to hybridize with the H-2B C and the β_2 m probes (Fig. 3A, lanes b-d; results shown with probes B and D) even with autoradiographic exposure for 2 weeks (data not shown). These poly(A)⁺ RNAs were considered as undegraded because they hybridized strongly with an actine probe, yielding an intense band with an apparent size of 2,000-2,100 nucleotides and no smear (Fig. 3B). When H-2 and β_2 m probes, displaying a much higher specific radioactivity (2× 10° c.p.m. per μg) were used, faint bands were observed or poly(A)+ RNAs extracted from EC cells, at the same location as in adult cells. No additional bands were observed on the latter control (Fig. 3C, lanes a, c). By comparison of the intensities of the bands obtained with similar amounts of poly(A)+ RNA prepared from EC and adult cells, we conclude that the former contained about 100 times less H-2- and β_2 m-specific mRNA than the latter. This result can be accounted for by either a normal expression in about 1% of the cells or by a 1% expression in most of them. Serological data¹¹ favour the first hypothesis.

Hybridization to H-2 and β₂m probes was carried out using poly(A)+ extracted from differentiated cells derived from EC lines by in vitro differentiation. We observed the same pattern as with poly(A)+ extracted from adult organs: one band migrating as ~1,800 nucleotides hybridizing with H-2 probe and two bands migrating approximately as 1,000 and 800 nucleotides hybridizing with β_2 m probe. The intensity of the bands was dependent on the relative amount of H-2 antigens expressed by the cells tested: faint bands are observed with mRNAs prepared from 3/A/1-D-3 (ref. 17), a fibroblastic line derived from PCC3 exhibiting a low amount of H-2 antigens (data not shown). The intensity of the bands observed with mRNAs prepared from C17-S1-D-T 984 (ref. 23), a muscle cell line isolated from a contractile zone of cultured cells derived from a C3H teratocarcinoma, indicates that there is about the same amount of specific H-2 and β_2 m mRNA in these cells as in adult cells (Fig. 3C, lanes b, c), confirming the immunofluorescence data.

These results suggest that there is about 1% of H–2 and β_2 m poly(A)⁺ RNA in EC cells as compared with adult cells. Interestingly, this applies to all members of the H–2 multigene family^{14,24}, as well as to any other mRNA which could contain the reiterated sequence detected with probe H–2C. Moreover, our results rule out the possibility that H–2 heavy chains are synthesized in EC cells and remain undetected at the cell surface through lack of β_2 m, as reported in several cell lines such as the human Daudi cells²⁵.

To determine whether major rearrangements at the DNA level would account for the absence of H-2 and β_2 m expression in EC cells, we have carried out Southern blots using DNAs extracted from F9, PCC4 EC cells and from 129/Sv liver. DNAs were digested with BamHI and EcoRI, run in 0.7% agarose gels, transferred to nitrocellulose sheets and hybridized with H-2B and β_2 m probes. The multi-band patterns obtained with probe H-2B on EC and liver DNAs are identical as far as the sizes of the bands are concerned; however, differences in the intensity of several bands do exist (Fig. 4). Two major bands were detected when using the β_2 m probe, and their sizes did not change on differentiation (data not shown). These findings confirm the absence of major DNA rearrangements in and around β_2 m and H-2 genes during differentiation 26 .

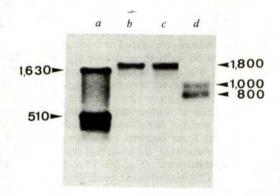
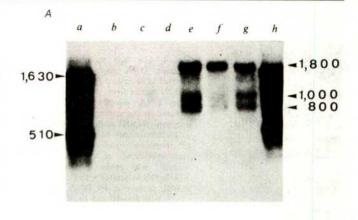


Fig. 2 Poly(A)⁺ RNAs were extracted from adult organs, precipitated with ethanol and isolated by binding twice to oligo(dT) cellulose as described elsewhere³¹. 5 μg of each preparation were subjected to electrophoresis in a 1.1% agarose gel and transferred to nitrocellulose sheets, also as described elsewhere¹⁸. The sheets were prehybridized, hybridized to ³²P-radio-labelled restriction fragments. A, B, C and D restriction fragments (Fig. 1) were polymerized using T4 DNA ligase (Boehringer Mannheim) and then ³²P radiolabelled by nick-translation³² to a specific activity of 2–3× 10⁸ c.p.m. per μg. Each nitrocellulose sheet was hybridized with 10⁷ c.p.m. of each probe for 24 h and then washed as described previously¹⁸. The sheets were then autoradiographed on flash-activated Kodak X-Omat films for 2–14 days depending on experiments, at −70 °C with intensifying screens. Lane a, 2 ng of pBR322 digested by *Hin*fl were hybridized with a pBR322 probe. Lanes b, c, 5 μg of poly(A)⁺ RNA respectively extracted from DBa/2 liver and from 129/Sv liver, spleen and kidney, were hybridized with probe B. Lane d, 5 μg of poly(A)⁺ RNA extracted from liver DBA/2 were hybridized with probe D. (Autoradiographic exposure time 48 h.) In our experimental conditions, 18S rRNA migrates slightly above 1,800 bp (a location corresponding to a size of 1,920 bp DNA).



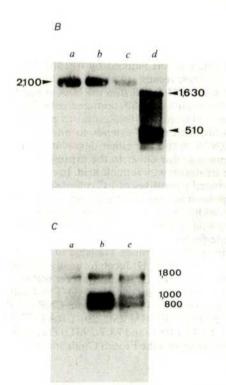


Fig. 3 A, Poly(A)⁺ RNAs were extracted from EC cell lines and used for Northern blotting as described in Fig. 2 legend. Lanes a, h, 10 ng of pBR322 digested by Hinfl were hybridized with a pBR322 probe. Lanes b-d, 5 μg of poly(A)⁺ RNAs respectively extracted from PCC4-aza, F9 and PCC3 EC cell lines, were hybridized with probes B and D. Lanes e-g, 5 μg of poly(A)⁺ respectively extracted from DBA/2 liver, DBA/2 spleen and 129/Sv liver, spleen and kidney, were hybridized with probes B and D. (Autoriadiographic exposure time 48 h.) B, Lanes a-c, 5 μg of poly(A)⁺ RNAs, respectively extracted from PCC3, F9 and PCC4-aza, were hybridized with an actine probe. Lane d, 2 ng of pBR322 digested by Hinfl were hybridized with a pBR322 probe. (Autoradiographic exposure time 48 h.) C, Lane a, 10 μg of poly(A)⁺ RNA extracted from PCC4-aza EC cells. Lane b, 4 μg of poly(A)⁺ RNA extracted from 129/Sv liver, spleen and kidney. The nitrocellulose filter was hybridized simultaneously with B and D probes. (Autoradiographic exposure 24 h.)

It thus seems likely that H-2, H-2-related genes and the β_2 m gene(s), taken here as developmentally regulated genes, are primarily controlled at the level of transcription. These genes belong to different chromosomes (H-2, TL, Qa genes, lie on chromosome 17, β_2 m probably maps on chromosome 2^{27} ; the locations of H-2-related genes are not precisely known). Therefore, no common *cis*-regulatory process could be postulated on a topological basis.

Croce et al.²⁸ published similar but not identical results on F9 EC cells transformed with a plasmid carrying herpes simplex virus type 1 TK gene and SV40 genes²⁹. The expression of H-2

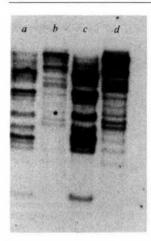


Fig. 4 The high molecular weight genomic DNA was extracted and purified as described previously24 from 129/Sv mouse liver and from F9 EC DNA samples (20 μg) were digested by the appropriate restriction enzymes (BamHI or EcoRI), subjected to electrophoresis in a 0.7% agarose gel, transferred to nitrocellulose sheets and hybridized as described previously24 with probe B. Lanes a-b, 129/Sv DNA digested with BamHI and EcoRI; lanes c-d, F9 DNA digested with BamHI and EcoRI.

and SV40 antigens was induced by treatment of the cells by retinoic acid. Their results are identical to ours concerning the absence of specific H-2 and β₂m mRNAs in nondifferentiated cells, but their results on differentiated cells differ from ours. Croce et al. obtained a hybridization to a smear and not to distinct bands. This smear extends to much lower molecular weight mRNAs, suggesting either degradation or alteration of a normal message due either to the expression of the inserted plasmid or treatment with retinoic acid. In contrast, clonal lines of differentiated derivatives of EC cells display, in our studies, the same pattern as normal differentiated cells. Thus, the pattern observed by Croce et al. might reveal changes in a normal gene expression caused by a non-physiological differentiatory agent or interference with viral genes.

The actine probe was made available to us by F. Brégégère and A. Minty. We thank H. Jacob for many helpful discussions and the gift of EC cells, D. Rocancourt for technical assistance and M. Caravatti for his gift of C17-S1-D-T 984 poly(A)⁺ RNA. This work was supported by grants from CNRS (ER 201, ATP 4246 and 5039), INSERM (SC 20, CRAT 72.79.104) and DGRST (ATP 79.7.0500 and 78.7.2931). The work was carried out in accordance with the French Guidelines for Recombinant Research.

Received 26 October 1981; accepted 26 January 1982

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Nuclear localization and DNA binding of the transforming gene product of avian myelocytomatosis virus

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Oncornaviruses transform cells either directly through a viruscoded oncogene¹ or, if they lack such a gene, indirectly by promoter insertion²⁻⁴ into the cellular genome and activation of a cellular gene. Both transformation mechanisms ultimately result in abnormally high expression of normal genes. Recently, the activated cellular gene in certain lymphomas4 has been shown to be homologous to the oncogene of an acute avian leukaemia virus, MC29. In MC29 the oncogene is fused to the viral structural gene, gag. The product of this fused gene is a protein of molecular weight 110,000 (110 K)5-7, designated p110gag-myc. We have characterized this protein by using monoclonal antibodies against p19, the N-terminal portion of the gag-myc fusion or 110 K protein and purified it 3,700-fold by immune affinity column chromatography. Immunofluorescence studies and cell fractionation of MC29-transformed fibroblasts indicate that the 110 K protein is predominantly located in the nucleus. Moreover, the purified protein binds to doublestranded DNA. These properties may be related to the role of the protein in transformation.

The MC29-transformed quail embryo fibroblast cell line. MC29-Q8-NP, does not produce virus particles but expresses the transforming protein gag-myc, which is fused to the viral structural gag proteins p19 and p27⁵. The monoclonal antibody against p198 allowed immunoprecipitation of the 110 K protein (Fig. 1A). Superinfection of this cell line with a helper virus, Rous-associated virus RAV-60, resulted in synthesis of viral polyprotein precursors which were about five times more abundant than the 110K protein. RAV-60-infected quail fibroblasts exhibited viral polyprotein precursors but no 110K protein (Fig. Indirect immunofluorescence using the IgG of monoclonal anti-p19 and fluorescein-conjugated anti-mouse IgG9 revealed that the MC29-Q8-NP cells showed a strong nuclear fluorescence (Fig. 2a, d) while the helper virus-superinfected cells showed a predominantly cytoplasmic staining (Fig. 2b). Normal quail embryo fibroblasts did not give rise to a positive fluorescence (Fig. 2c), while predominantly cytoplasmic fluorescence was seen in quail embryo fibroblasts infected with RAV-60 (Fig. 2e) and in the Rous sarcoma virus-transformed cell line R(-)Q, which expresses the transforming protein pp60^{src} and viral polyprotein precursors¹⁰ (Fig. 2f). Therefore, the nuclear fluorescence of MC29-transformed quail fibroblasts seems to be specific for transformation by MC29. All other known oncornaviral transforming proteins were shown to be localized mainly in the cytoplasmic membrane¹¹

To confirm our observation, a cell fractionation from radioactively labelled MC29-Q8-NP cells and RAV-60-superinfected MC29-Q8-NP cells was performed. Nuclei were isolated by hypotonic shock and Dounce homogenization and subsequently stripped by treatment with nonionic detergent12, a procedure which leaves behind a crude chromatin preparation. The soluble fractions were combined and contained endoplasmic reticulum, organelles, cytoplasmic and nuclear membranes and polysomes. The stripped nuclei contained 70% of the 110K protein, while the residual 30% were present in the soluble fraction, which also contained >90% of each of the viral proteins Pr180gag-pol, Pr76gag, p27, Pr92env and gp85. These values were derived from the radioactivity eluted from the various protein bands of the gel shown in Fig. 1B and C and corrected

for differences in volumes. The 110K protein was only detectable in the soluble fraction if 10 times more radioactive material was used (Fig. 1B).

For further analysis the 110K protein was purified from 35S-methionine-labelled MC29-Q8-NP cells by an immune affinity column containing protein A-Sepharose to which monoclonal anti-p19 IgG was covalently coupled in conditions which link the constant region of the IgG molecule to the solid phase 13. The 110K protein was eluted from the column with a buffer of low pH (pH 2) and salt (Fig.3a), conditions which have been applied to purify biologically active human leukocyte interferon 14. Comparison of the radioactive material eluted from the immune affinity column with the total radioactive input indicated a 3,700-fold purification. The eluted 110K protein was precitable by anti-p19 serum, indicating that it was still antigenically active (Fig. 1, slots a, b). The p19-containing viral polyprotein precursors in virus-producing cells were isolated by the identical purification procedure from Rous sarcoma virus-infected chicken embryo fibroblasts (Fig. 3b). Many p19related polyproteins were eluted, the majority of which was represented by Pr76gag, which served as a control for nucleic acid-binding studies described below.

Because the localization of the 110K protein in the nucleus raised the question of whether it interacted with cellular DNA, the purified protein was analysed for its nucleic acid-binding properties in vitro. DNA of a defined size class was preparatively isolated from avian fibroblasts¹⁵, mixed with the ³⁵S-methionine-labelled purified 110K protein and sedimented in a glycerol gradient. In these conditions the 110K protein comigrated with the DNA, whereas without DNA it remained on top of a gradient run as a control (Fig. 4a). To determine whether the nucleic acid-binding property of the 110K protein was attributable to the gag or the transforming protein portion, Pr76gag and p19-related polyproteins were analysed in the same way. These proteins did not bind to the DNA (Fig. 4b), indicating that it is the transformation-specific moiety of the 110K protein which interacts with the DNA.

The nucleic acid-binding property of the 110K protein was also demonstrated by using radioactively labelled DNA to measure the ability of the 110K protein to retain DNA on a nitrocellulose filter in a filter binding assay¹⁶. The 110K but not the Pr76gaag protein allowed the binding of DNA quite efficiently (Fig. 4c). Use of ³⁵S-methionine-labelled proteins enabled us to control the linearity of binding of the protein to

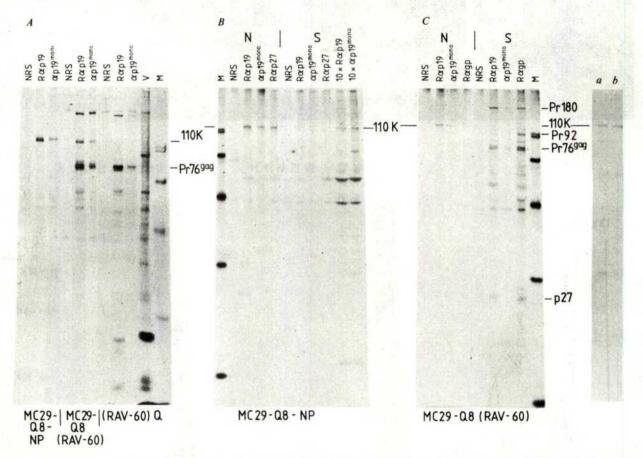


Fig. 1 Distribution of 110K protein in cells and subcellular fractions. *A*, MC29-Q8-NP cells, MC29-Q8 cells superinfected with RAV-60 (MC29-Q8 (RAV-60)) and RAV-60-infected quail fibroblasts ((RAV-60)Q) (3×10⁶ cells each) were labelled with ³⁵S-methionine (250 μCi ml⁻¹) for 4 h, lysed and treated with anti sera and *Staphylococcus aureus*²¹ for indirect immunoprecipitation. The precipitates were analysed on polyacrylamide slab gels and processed for autoradiography²⁰. The sera used were: NRS; normal rabbit serum; Rαp19, rabbit anti-p19; αp19^{mono}, purified IgG of monoclonal antibodies against p19; 5 μl of each serum was used and 10 μg of the IgG. V represents ³⁵S-methionine-labelled RAV-60 virus and M a set of ¹⁴ C-labelled marker proteins (Amersham) (from top) molecular weights of 92.5K (upper band of doublet), 69K, 46K, 30K and 14K. *B*, Isolation of stripped nuclei was performed according to published procedures ¹². MC29-Q8-NP cells (1.5×10⁷) were radioactively labelled with 250 μCi ml⁻¹ of ³⁵S-methionine²⁰ for 4 h and processed immediately. The plates were washed three times with ice-cold phosphate-buffered saline (PBS, 17 mM Na₂HPO₄, 2.6 mM KH₂PO₄ pH 7.4, 120 mM NaCl) and treated with hypotonic buffer (10 mM Tris-HCl pH 7.3, 10 mM NaCl, 1.5 mM MgCl₂) for 40 min at 4°C for lysis. Subsequently the cells were homogenized in a tightly fitting Dounce homogenizer with 10-20 strokes. Release of nuclei was followed by phase contrast microscopy. The nuclei were pelleted at 3,000 r.p.m., for 5 min, and the pellet was resuspended in hypotonic buffer and treated with 1% NP40 and 0.5% desoxycholate (DOC) to strip the nuclei. After mixing on a Vortex for 4 s the stripped nuclei (N) were pelleted (3,000 r.p.m., 5 min). The soluble supernatants (S) were combined and brought up to 50 mM Tris-HCl pH 7.2, 150 mM NaCl, 0.1% SDS, 1% DOC, 1% Triton X-100 (RIPA buffer). The stripped nuclei were suspended in 1 ml RIPA buffer. After incubation for 15 min at 0°C, samples were centrifuged (10,000

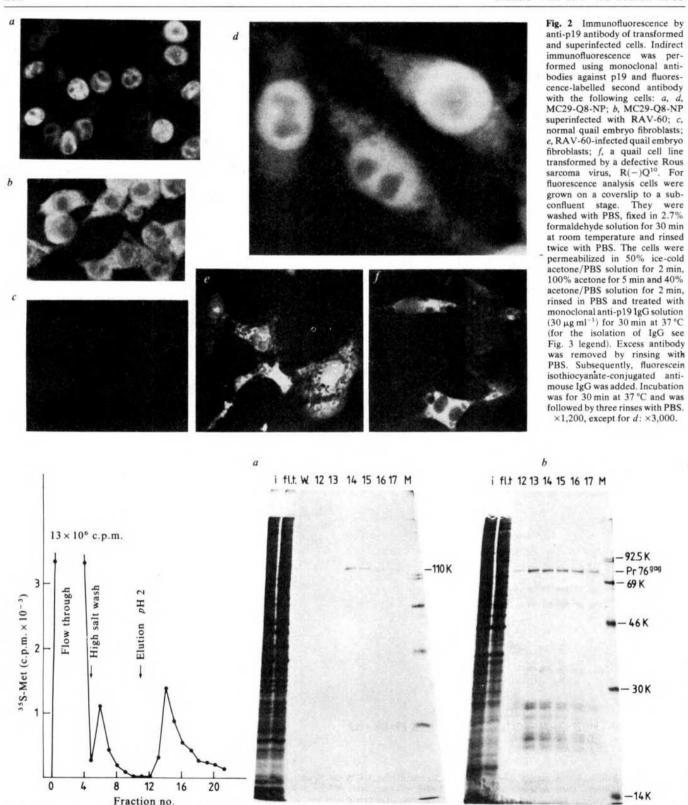
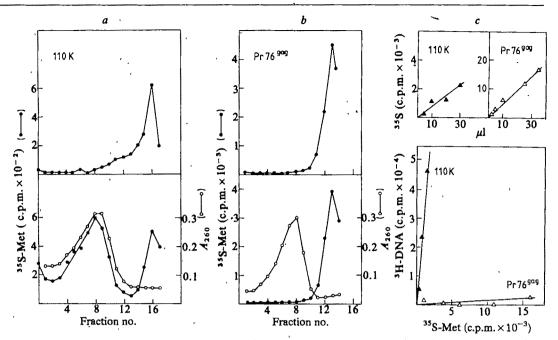


Fig. 3 Isolation of 110K protein from MC29-Q8-NP cells and Pr76^{gag} from virus-producing chicken fibroblasts by immune affinity chromatography. *a*, IgG from monoclonal anti-p19 was purified by protein A-Sepharose column chromatography (Pharmacia) from hybridoma supernatant and eluted with 4 M MgCl₂. The immunoabsorbance column was prepared according to Gersten and Marchalonis¹³: 7 mg of purified anti-p19 IgG were covalently coupled to 1 ml of protein A-Sepharose CL-4B by dimethylsubermidate (30 mg) in 0.1 M borate buffer, *p*H 8.0. Then the column was pretreated successively with 1 ml each of PBS, PBS plus bovine serum albumin (1 mg ml⁻¹), PBS, a lysate of 10⁸ normal quaii methyo fibroblasts, and PBS. For preparation of the cell lysate, 5×10⁷ MC29-Q8-NP cells were labelled with 250 μCi ml⁻¹ of ³⁵S-methionine for 4 h (ref. 20), lysed with 10 ml RIPA buffer in the presence of trasylol (Bayer) and centrifuged at 10,000 r.p.m. at 4 °C for 30 min. The supernatant (12 ml containing 6.4×10⁸ c.p.m.) was then applied to the immune affinity column (200 μl bed volume). The column was washed successively with 5 ml RIPA buffer, 5 ml high-salt buffer (20 mM Tris-HCl *p*H 7.5, 1 M NaCl, 0.5% NP40) and 2 ml PBS before the protein was eluted with a buffer containing 0.1 M citric acid *p*H 2, 300 mM NaCl, 50% ethylene glycol, 0.1% Triton X-100. Fractions of 400 μl were collected, 20 μl of each fraction was counted in a scintillation counter and 50 μl applied to a SDS-polyacrylamide slab gel (10%). In total, 1.8 × 10⁵ c.p.m. were eluted, indicating a 3,700-fold enrichment. The 110K protein-containing fractions were diluted and dialysed overnight against 50 mM phosphate buffer *p*H 6.2, 100 mM NaCl, 1 mM dithiothreitol and 10% glycerol. i, 5 μl of the input (12 ml); fl.t., 5 μl of the flow-through; W, 5 μl of the pooled wash (10 ml); 12-17, eluted fractions; M, ¹⁴C-labelled marker proteins (see Fig. 1 legend). b, The identical procedure was performed with Rous sarcoma virus-infected chicken embryo fibroblast

Fig. 4 Interaction of the 110K protein with DNA. a, DNA was extracted from chicken embryo fibroblasts15 and sheared by pressing it six times through a 21-gauge needle. The DNA was centrifuged through a 10-30% glycerol gradient in a SW41 rotor at 40,000 r.p.m. for 5 h, and the peak fraction was premethionine-labelled dialysed protein from immune affinity column (fractions 14 and 15, Fig. 3a). The protein was mixed with the DNA (50 µg in 50 µl) and meubated at 30 °C for 10 min in 50 mM Tris-HCl pH 8, 100 mM NaCl, 2 mM EDTA buffer. The reaction product was sedimented through a 10-30% glycerol gradient in the same buffer. Centrifugation was in a SW41 Tr rotor at 30,000 r.p.m. for 19 h at 4 °C. 500 µl fractions were collected and 10 µl per fraction was used to measure the radioac-



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tivity. The DNA content of each fraction was determined by measuring the absorbance at 260 nm. The control gradient contained only the protein and no DNA. b, To analyse the interaction of Pr76^{prac} with DNA, the same procedure was applied. Pr76^{prac} and other p19-related proteins golated by immune affinity chromatography (see Fig. 3b) were dialysed and sedimented in the same conditions as the 110K protein in the absence of DNA. c, Filter binding assay of 110K and Pr76^{prac} proteins and DNA The ability of the purified 110K and Pr76^{prac} proteins (fraction 14, Fig 3a and b) to bind to DNA were analysed using a filter binding assay¹⁶. The incubation mixture (0.5 ml) contained 50 mM Tris-HCl PH 8.0, 2 mM EDTA, 2 µg of sheared ³H-labelled double-stranded DNA from chicken embryo fibroblasts, corresponding to 155,000 c.p.m., and proteins as indicated. After 10 min at 30 °C, the mixture was slowly filtered through a nitrocellulose filter (Selectron, Schleicher & Schuell, type BA85) and washed with 10 ml of the buffer used in the incubation mixture. The filter was pretreated with 0 3 M NaOH for 5 min and washed with H₂O (ref. 22). Binding of the proteins to the filter was controlled by determination of the ²⁵S-methionine radioactivity bound to the filters (top).

the filter (Fig. 4c, top). From this assay it was estimated that $\sim 1~\mu g$ of 110K protein bound to 1 μg of cellular DNA, based on specific activities of 5×10^6 c.p.m. per mg of cellular proteins and 8×10^4 c.p.m. per μg of DNA.

To rule out the possibility that binding of the 110K protein to DNA was a consequence of the stringent elution conditions from the immune affinity column and not a property of the native protein, DNA binding to 110K was analysed by a modified approach. Instead of eluting the protein from the immune affinity column described in Fig. 3, the loaded column was exposed to 3 H-labelled cellular DNA. In these conditions 85% of the DNA was bound to the column and did not elute with 0.6 or 2 M NaCl; only treatment with 1% SDS allowed its elution (Fig. 5a). A subsequent treatment of the column with pH 2 buffer removed the 35 S-methionine-labelled 110K protein. When the column was then analysed in a control experiment for its binding of DNA without the 110K protein, most of the DNA did not bind in these conditions (Fig. 5b).

We describe here for the first time the isolation and characterization of a transformation-specific protein of an acute avian leukaemia virus. It differs from other known oncornaviral proteins¹¹ in its nuclear localization and ability to bind to DNA. None of the gag-related polyproteins shown in Fig. 3b bound to DNA, indicating that DNA binding is probably due to the

transforming part. However, the biological significance of this property is unknown. Furthermore, transformation of fibroblasts analysed here will have to be compared with that of bone marrow cells, the *in vivo* target cells which differ in their transformation parameters¹⁷. The properties of 110K protein—although novel for oncornaviral transformation-specific proteins—closely resemble those of the large T antigen of SV40¹⁸.

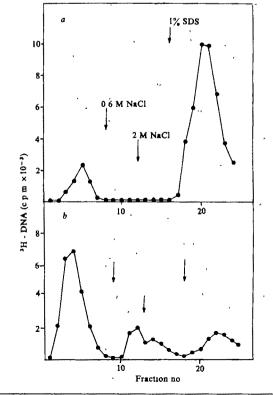


Fig. 5 Interaction of DNA and immune affinity column-bound 110K protein. a, An immune affinity column was prepared and loaded with 35 S-methionine-labelled cellular lysate as described in Fig. 3 legend. Instead of eluting the 110K protein from the column, 3 H-labelled cellular DNA ($^{7.5} \times 10^{5}$ c.p.m., specific activity 105 c.p.m. per μ g) was loaded onto the column. A wash with PBS removed 15% of the DNA from the column. It was then treated with 0.5 ml TE buffer (50 mM Tris-HCl pH 8.0, 2 mM EDTA) containing 0.6 M NaCl and 2 M NaCl, respectively. No DNA was eluted. Only with TE buffer containing 1% SDS, 85% of the total DNA mput radioactivity was recovered. 0.1-ml fractions were collected, 5 μ 1 of each fraction being used to determine the radioactivity. The column was then treated with μ 1 2 buffer to elute the 110K protein as described in Fig. 3a legend. Subsequently the column was rinsed with PBS and used for a control experiment (b). The same amount of 3 H-labelled DNA was applied to the column and treated exactly as described in a. 65% of the input DNA appeared in the flow-through, 25% eluted with 0.6 M and 2 M NaCl and residual 10% with 1% SDS.

Other gag-onc fusion proteins can be isolated in the way described here and their properties compared. Also, the availability of the purified 110K protein will permit polyvalent or monoclonal antibodies to be raised—preferably after removal of the gag portion by p15^{19,20}—so that transformation-specific antibodies will become available.

We thank M. K. Owada, T. Bunte and Erich Lanka for stimulating discussions and cooperation, and Klaus Bister for supplying the MC29-Q8-NP cells.

Received 5 November 1981; accepted 29 January 1982

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Spontaneous fragmentation of actin filaments in physiological conditions

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Actin filaments have been shown to result from nucleation and consecutive binding of actin monomers to the ends of filaments1. Nucleation is assumed to consist of the aggregation of a few monomers to form a small filament, but apart from the nucleation and the elongation reaction, end-to-end association of filaments has also been reported2. It is not known whether in physiological conditions actin filaments can break spontaneously at subunit contacts along the filament. In view of the possible importance of spontaneous fragmentation in the determination of the length, number and turnover of thin filaments in the cell, I have now studied this reaction by measuring the polymerization kinetics of actin at low total concentrations in physiological conditions (1 mM MgCl₂, 100 mM KCl, 37 °C). Quantitative analysis of the polymerization curves suggests that actin filaments break spontaneously and consequently create more nucleation sites as the reaction proceeds. This explains the autocatalytic nature of the polymerization kinetics.

The polymerization was followed by 90° light-scattering intensity using a Zeiss fluorometer (546 nm). The light-scattering intensity R of long rod-like aggregates such as actin filaments has been shown to be proportional to the concentration of actin filament subunits c_i (refs 3,4).

$$R = a \cdot c_t \tag{1}$$

The constant a was calibrated by measuring the scattering intensity of solutions of known actin filament subunit concentrations. Actin was prepared as described earlier⁴, thereby omitting the modification with N-ethylmaleimide, and the protein was applied to a Bio-Gel P-150 column to remove aggregates. Actin (<10 μM) was dialysed against a buffer containing 0.5 mM ATP, 10 µM MgCl₂, 1 mM dithiothreitol, 200 mg l⁻¹ NaN₃ and 5 mM triethanolamine hydrochloric acid (pH 7.5). The polymerization was initiated by mixing two volumes of dialysed monomeric actin with one volume of a buffer consisting of 0.5 mM ATP, 1.5--6 mM MgCl $_2$, 150--600 mM KCl , $0\text{--}150~\mu\text{M}$ CaCl₂, 0-600 µM EGTA, 10 mM dithiothreitol, 200 mg l NaN₃ and 5 mM triethanolamine hydrochloric acid (pH 7.5). Before mixing, all solutions were centrifuged for 1 h at 100,000g to remove dust and possibly occurring actin polymers. All experiments were performed at 37.1 °C.

The polymerization curves are depicted in Fig. 1. An attempt was made to fit these curves by a simple polymerization model⁴ that takes into account nucleation $(K_N, equilibrium constant)$ for formation of a nucleus from n monomers), association of monomers to the filament ends (k, association rate constant) and dissociation of subunits from the filament ends (k', dissociation rate constant). However, it was not possible to find calculated curves that were in good agreement with measured kinetics (Fig. 1). Particularly at low total actin concentrations, the measured curves are significantly more sigmoidal than the calculated curves.

The long lag phase observed at low total actin concentrations is indicative of an autocatalytic reaction such as self-reproduction of filaments by fragmentation. To test whether the experimental data are compatible with fragmentation of filaments, a kinetic theory can be readily developed by extension of the nucleation elongation model⁴. Filament subunits (concentration c_1) result from binding of monomers (concentration c_1) to the ends of filaments and are consumed by dissociation of subunits from the ends. Hence

$$\frac{dc_t}{dt} = (kc_1 - k')C = (k(c_{tot} - c_t) - k')C$$
 (2)

where c_{tot} is the total actin concentration and C is the concentration of actin filaments. The rate of filament formation by

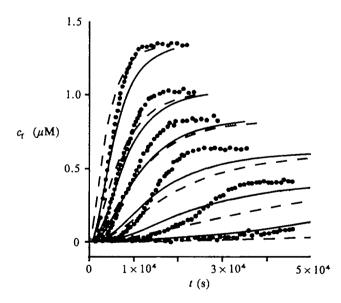


Fig. 1 Time course of the actin filament subunit concentration c_t. Salt concentrations: 1 mM MgCl₂ and 100 mM KCl. ● Measured kinetics, total actin concentrations (µM): 0.41, 0.62, 0.82, 1.03, 1.23 and 1.54; final constant monomer concentration (critical concentration): $\bar{c}_1 = k'/k = 0.21 \,\mu\text{M}$. , Calculated curves; the nucleus was assumed to be a dimer (n = 2) and fragmentation was assumed not to occur; rate parameter obtained by combining equations (2) and (3): $k^2 K_N = 1 \times 10^3 \text{ mol}^{-1} \text{ s}^{-1}$ Calculated curves; the nucleus was treated as a tetramer (n = 4)and fragmentation was assumed not to occur; rate parameter: $k'^2K_{\rm N}=1.1\times10^9\,{\rm mol}^{-3}\,{\rm s}^{-2}.$

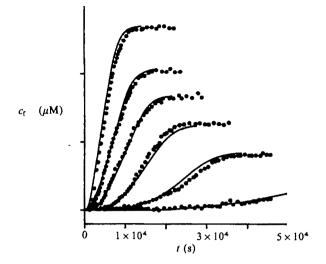


Fig. 2 Time course of the actin filament subunit concentration ct. For salt concentrations and actin concentrations see Fig. 1 legend. , Measured curves. , Calculated curves; the nucleus was treated as a tetramer (n = 4) and fragmentation was assumed to occur; rate parameters obtained by combining equations (2) and (3): $k'^2 K_N = 5.8 \times 10^8 \text{ mol}^{-3} \text{ s}^{-2}$, $k' k_t = 1.7 \times 10^{-8} \text{ s}^{-2}$.

nucleation and fragmentation is given by

$$\frac{\mathrm{d}C}{\mathrm{d}t} = (k(c_{\mathsf{tot}} - c_{\mathsf{f}}) - k')K_{\mathsf{N}}(c_{\mathsf{tot}} - c_{\mathsf{f}})^{n} + k_{\mathsf{fr}}c_{\mathsf{f}}$$
(3)

 k_{tr} is the rate constant of fragmentation at a subunit contact zalong an actin filament. The first term of the kinetic equation (3) represents nucleation from monomers⁴ whereas the second term gives the rate of filament formation by fragmentation. Because of the uniformly double-helical structure of actin filaments the fragmentation reaction is assumed to occur at any subunit contact with the same rate, thereby neglecting end effects. When fragmentation is assumed to occur, polymerization curves can be calculated that reveal a long lag phase at low total actin concentrations and are in good agreement with the measured data (Fig. 2).

Slow isomerization steps in the early phases of polymerization can be excluded as a reason for the lag phase because isomerization steps lead to a lag phase independent of the total actin concentration whereas the experimental curves have increased sigmoidicity only at low total actin concentrations.

Addition of calcium (50 µM) or EGTA (200 µM) or variations in the magnesium (0.5-2 mM) or potassium concentration (50-200 mM) did not significantly affect the shape of the polymerization curves. The experiments suggest that actin filaments can break spontaneously quite independently of the ion concentrations.

The inherent instability of actin filaments resulting in spontaneous fragmentation may provide a further pathway of filament formation in addition to nucleation from actin monomers and growth of filaments induced by other molecules. This reaction may be important in determining the length and the number of filaments in the cell. Recently, several molecules^{5,6} have been reported to cause the conversion of filaments into short fragments by binding to one end of the filaments. The question arises of whether these molecules act by preventing end-to-end association of spontaneously broken filaments or whether they disrupt actin filaments.

Received 13 November 1981, accepted 29 January 1982

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The C conformation is a low salt form of sodium DNA

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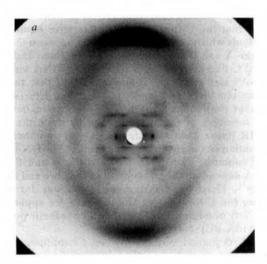
Three well-defined conformations have been observed for naturally occurring DNAs in oriented fibres. These have been designated A, B and C and their detailed geometries in a variety of crystalline and semi-crystalline forms determined by X-ray fibre diffraction analysis¹⁻⁵. The C conformation is commonly observed as a low humidity form of the lithium salt3. However, when the counter-ion is Na⁺ rather than Li⁺, previous X-ray fibre diffraction studies^{6,7} have suggested that the C form is at best a poorly favoured conformation. Arnott and co-workers^{6,2} reported that the C form is stable at relative humidities and salt contents which are both intermediate between those which favour the A and B conformations. Zimmerman and Pheiffer9 observed C-like patterns from fibres of Na-DNA immersed in t-butanol/water mixtures. They emphasized that the C form should be visualized as a family of related structures and suggested that continuous smooth transitions occur between the various members of the C family and between the B and C forms. IR linear dichroism studies have shown that a C-like conformation can occur in oriented films of Na-DNA¹⁰ at very low salt contents. Transitions between the A and B forms of Na-DNA depend on the salt content of the fibre and its relative humidity11. Here we describe the conditions for routinely observing the C form of Na-DNA. These are applicable to a wide variety of natural DNAs and to the synthetic polynucleotide poly(dA-dC) · poly(dG-dT).

The DNAs studied were calf thymus, Clostridium perfringens. herring sperm, pollock roe, salmon sperm, Escherichia coli (all from Sigma), SP 15 (from Professor M. Mandel), T2 phage, Micrococcus lysodelkticus (both from Miles), $\phi W-14^{11}$, and poly(dA-dC) · poly(dG-dT). The final stage in the preparation was by either ethanol precipitation from ≤0.1 M NaCl or centrifugation at 50,000 r.p.m. from NaCl as low as 0.001 M. Fibres were drawn from either the precipitate or the concentrated gel and X-ray diffraction patterns obtained at relative humidities from 0 to 98%. The NaCl concentration in a fibre was increased by re-dissolving it in a small quantity of distilled water and then adding to it a small quantity of a standard NaCl solution. A new fibre was pulled from the resulting gel, without loss of DNA. When fibres were made from precipitated material, the piece used was weighed and the DNA in the specimen determined after allowing for water content. For specimens from centrifuged material similar estimates were made using measurements of A_{260} for the original solution and supernatant. Hence, it was possible to calculate the number of Na⁺ and Cl⁻ added per DNA PO₄.

All the Na-DNAs studied could be induced to assume the C form and Fig. 1 shows examples of their diffraction patterns. Where the patterns were sufficiently well defined they were of the hexagonal rather than orthorhombic type, with helical parameters not significantly different from those previously reported³. Conditions were found for which all the DNAs studied assumed the B conformation and also, with the exception of DNA from T2 and SP15, the A conformation. C patterns from the various Na-DNAs were only observed from fibres

containing very little NaCl and in an environment of low relative humidity.

For all DNAs which could assume the A form, fibres giving the C pattern at low humidity exhibited transitions first from the C form to the fully crystalline A form and then from A to the semi-crystalline B form as the relative humidity was increased. Generally, the greater the NaCl content of the fibre the lower the relative humidity at which the A pattern occurred. For the lowest amounts of NaCl, C was usually observed at relative humidities from 32 to 75% but as the NaCl content was increased the upper limit in the relative humidity at which C occurred was reduced until it reached a level when the C pattern was no longer observed. For poly(dA-dC) · poly(dG-dT) this occurred when more than 0.7 Cl ions per DNA PO₄ had been added to the fibre, but for natural DNAs rather less NaCl was required—typically 0.5 Cl⁻ per PO₄. Below 32% relative humidity the X-ray diffraction patterns became much less well defined, indicating a progressive collapse of the structure. As reported previously¹¹, the relative humidity at which the A to B transition occurred decreased with increasing NaCl content. Generally, the transitions $C \rightarrow A \rightarrow B$ were fully reversible as the relative humidity was reduced. However, for poly(dAdC) · poly(dG-dT), with the NaCl concentrations so far studied, the A→B transition was reversible but the C form was only regained if the fibre was re-wetted to form a gel and a new



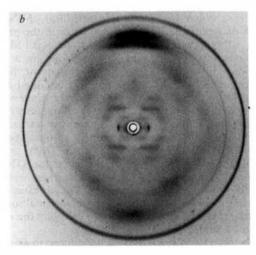


Fig. 1 X-ray diffraction patterns of the C type from calf thymus Na-DNA at 57% relative humidity (a) and Na poly(dA-dC)·poly(dG-dT) at 57% relative humidity (b) (the diffraction ring at 2.81 Å is from NaCl crystallites in the fibre). The two features generally regarded as characteristic of C patterns³ are a peak at ~ 0.1 Å $^{-1}$ on the first layer line and strong diffraction close to the meridian on the eighth layer line. The peaks on the eighth and ninth layer lines in b are particularly well resolved.

fibre drawn. For DNAs which undergo the $C \rightarrow A \rightarrow B$ transitions we have observed patterns which are mixtures of A and C and of A and B. DNA from T2 and SP15 underwent a transition from C to semi-crystalline B with increasing relative humidity.

These results establish the C form as a major conformational possibility for the DNA double helix in biological environments. In contrast to previous work, we have shown that there is no difficulty in inducing Na-DNA to assume the C conformation if both the water content and the level of NaCl in the fibre are sufficiently low. *In vivo*, DNA can be expected to occur predominantly either as the sodium salt or in a state where the Na⁺ ions are displaced by basic groups such as polyamines and lysine and arginine side chains in proteins. Situations can therefore be envisaged in which the conformation of an active region of DNA is controlled by changes in its hydration and the concentration of Na⁺ and Cl⁻ ions in its vicinity.

Earlier reports suggested that the C form of DNA was a variant of the B conformation, with ready transition between the two forms. While such a transition is observed for Li-DNA, in this study of Na-DNA the C → B transition passes through an intermediate A conformation except for those DNAs that do not assume the A form. In contrast to the earlier claim⁶ that at low hydration the NaCl content of fibres of Na-DNA which gave the C form was intermediate between those appropriate for observing the A and B forms, we find that C is favoured by NaCl contents lower than those which favour A. Our view of the A form as an intermediate between the C and B forms of Na-DNA is based on two distinct sets of observations. First, the $C \rightarrow A \rightarrow B$ transitions occur as a function of relative humidity for fibres containing small amounts of NaCl. Second, the $C \rightarrow A \rightarrow B$ transitions occur at a fixed relative humidity (say 57%) as the NaCl content of a fibre is raised.

A striking feaure of this study is the wide range of natural DNAs for which the C form was observed, particularly for DNAs with a G+C content of 31-72%, in agreement with IR studies10. Perhaps of even greater significance, the C form was observed for three DNAs (\$\phi\$W-14, SP15, T2) in which a substantial fraction of the nucleotides were extensively modified12-14. The observation of the C form for this very wide range of natural DNAs suggests it is a structure sufficiently favoured stereochemically for it not to be destabilized by substantial changes in either its charge distribution or the addition of bulky groups. We did not observe the A form for T2 DNA, in accord with earlier studies which suggested this was a consequence of glucosylation of a large fraction of the cytosine residues15. That report also suggested that at low humidity T2 DNA adopts a novel conformation, the T form. In view of our observation of the C form as a low humidity conformation of T2 DNA and because the helix pitch and overall intensity distribution reported for the T form are broadly similar to those which characterize C patterns, we believe that the T form is a close relative, if not a member, of the C family of structures.

The various synthetic polynucleotides exhibit much more variety in the conformations they assume than do natural DNAs8. We have therefore concentrated here on the occurrence of the C conformation for the sodium salt of naturally occurring DNAs. However, we have also studied the synthetic polynucleotide poly(dA-dC) · poly(dG-dT) as an example of DNA with an 'average' nucleotide composition but a highly regular base sequence. Although the C form is also observed for fibres of this DNA containing low amounts of salt at low relative humidities, these fibres differ from those of natural DNAs in the irreversibility observed in the C→A transition. This is reproducible, having been observed for a number of different fibres and also be re-wetting the same fibre. This unusual behaviour of poly(dA-dC) · poly(dG-dT) may be a consequence of its particular nucleotide sequence, resulting in significantly different base-stacking contributions to the conformational energy, or to different patterns of solvent interactions around the DNA.

This work was supported by SERC (to W.F.) and NATO (to W.F. and R.A.J.W.).

Received 16 November 1981, accepted 28 January 1982.

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Transfection of cells from a xeroderma pigmentosum patient with normal human DNA confers UV resistance

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Xeroderma pigmentosum (XP) is a human genetic disorder characterized clinically by extreme photosensitivity of the skin and eyes and a high incidence of skin cancer. Cell lines derived from XP patients are very sensitive to UV irradiation, and it is thought that the biochemical lesion responsible for this sensitivity is a defect in excision repair of UV-induced DNA damage1. We report here that transfection of cells from an XP patient with DNA from normal human cells can produce cells resistant to UV damage. The resistant cells have presumably acquired a gene conferring UV resistance; this study represents the first step towards the isolation and characterization of the gene.

Skin fibroblasts of a XP patient, XP20S, were transformed by simian virus 40 (SV40) and established as a permanent cell line, XP20S(SV), by Takebe et al.2. XP20S belongs to the complementation group A and shows an extremely low level of unscheduled DNA synthesis after UV irradiation². XP20S(SV), which has the characteristics of a cultured XP cell as well as those of the parental skin fibroblasts (it was highly UV-sensitive), was used as the transfection recipient.

High-molecular weight DNA was extracted and purified from human cultured cells or from Escherichia coli K-12 by sodium lauryl sulphate-phenol treatment after digestion with pronase E and ribonuclease (ref. 3); 10-30 μg of each DNA were transfected to 7×10⁵ cells of XP20S(SV) using a modification of the calcium phosphate precipitation method of Wigler et al.4. The transfected cultures were then irradiated with UV light and the UV-resistant (UV') cells were scored by colony formation. The transfection and selection of UV cells are described in Table 1 and the results are shown in Table 1a. No colonies were detected after mock transfection ('control' in Table 1a), or after transfection with the self DNA of XP20S(SV) or with E. coli DNA. UV colonies were detected in the cultures transfected with DNAs from human cell lines of rhabdomyosarcoma, A204 (ref. 5), and embryonic lung fibroblasts, MRC-5 (ref. 6), the response being relatively dose dependent. The cells isolated from 10 individual colonies per dish showed UV-resistance equal to that of the DNA donors, A204 and MRC 5. Figure 1 shows the UV sensitivity of the transformed XP20S(SV) cells. However, note that 3-6% of the colonies were very small and consisted of <10 individual cells. The cells from these minute colonies did not survive after colony isolation, and seemed to



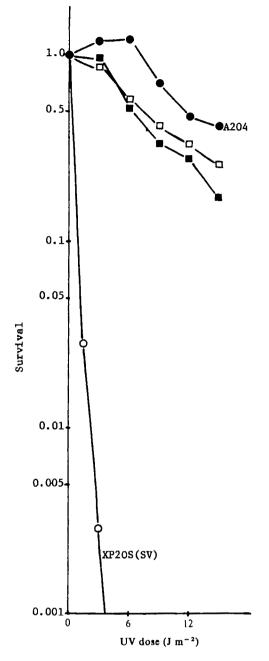


Fig. 1 UV-sensitivity of the transformed XP20S(SV) clones \Box , XP UV^r clone, 1; \blacksquare , XP UV^r clone 2), and the parental (O, XP20S(SV)) DNA donor cells (0, A204). In the experiment shown in Table 1a, colonies were isolated and 2×10^5 cells per colony were plated out in a 60-mm Petri dish, then irradiated with various doses of UV light from the bottom of the dish. The irradiated cells were cultured in the growth medium at 37 °C for 5 days, then the numbers of surviving cells were determined after trypsinization and Trypan blue staining. Results shown for XP UV clones 1 and 2 are typical of the cells of two representative colonies from the culture transfected with A204 DNA (20 µg per dish).

be practically UV-sensitive as were most of the recipient cells. Such minute colonies were detected neither in the mock infection nor in the dishes transfected by self and E. coli DNAs, which means that all the UV-sensitive XP cells were killed and UV transformants alone were detected in the experimental conditions used. The activity transforming the XP cell to UV was carried on the DNA molecules introduced into the transformed cells, because after treatment of A204 DNA with DNase I, there were virtually no UV^{r} transformants (Table 1a).

The frequency of the transformed UV colonies was 1.6-4.1 per µg DNA, whereas Wigler et al.4 described a transformation frequency of ~10 colonies per 20 µg of DNA for the thymidine kinase and adenine phosphoribosyltransferase genes. The higher transformation frequency in our study seemed to be due to cell growth during the 6-day incubation and to re-plating of the cultures before selecting the UV colonies. The numbers of cells in the transfected cultures increased ~4-5 times during the incubation and the generation time of XP20S(SV) was ~26 h in the culture conditions used. Therefore, the initial transformation frequency of XP20S(SV) to UV seems to agree with that obtained by Wigler et al.⁴. The UV-sensitive phenotype of XP20S(SV) was stable; we have never detected any UV colonies in control cultures (untreated) in the experimental conditions used. Together, these results demonstrate that the UV-resistance in transformants is derived from the donor DNA and is not due to reversion of the recipient cell.

It was of interest to determine whether XP20S(SV) could be transformed with DNA which had previously been digested with restriction endonucleases. This would be useful with regard to cloning the gene which renders the XP cell UV-resistant. We therefore analysed the sensitivity of the transforming activity of A204 DNA digested with various restriction enzymes recognizing different hexanucleotide sequences at their

Table 1 Transformation of XP20S(SV) cells to UV-resistance by DNA transfection

Donor DNA	Amount of transfected DNA per dish (µg)	DNA digestion before transfection	UV-resistant colonies per 7×10 ⁵ recipient cells
a			
A204	30	_	123
	20	-	75
	10	-	39
MRC-5	30	- ,	98
	20	-	80
	10	_	16
XP20S(SV)	30	_	0
E. coli	30	-	0
A204	30	DNase I*	0
Control	-	=	0
b			
A204	20	None	78
A204	20	SalI	97.2
A204	20	HmdIII	0 (9 2) [†])
A204	20	XhoI	142
A204	20	BamHI	0 (58 ^t)
A204	20	<i>Eco</i> RI	0
Control	-	-	0

The recipient cells, XP20S(SV), were plated at 7×10⁵ per 60-mm Petri dish. The next day, DNA from each donor cell was transfected by the procedure described elsewhere4. After 6 h incubation with the DNA-calcium phosphate precipitates, the cells were treated with 10% dimethyl sulphoxide in HBS4 for 30 mm at room temperature. Then the cells were washed once with HBS and incubated in Dulbecco's modified Eagle's minimum essential medium (Gibco) supplemented with 10% newborn calf serum (Mitsubishi Kasci Industrials) at 37 °C for 6 days. The culture medium was changed once a day. After incubation, the cultures were each trypsimzed and subdivided into four 60-mm Petri dishes (Lux, no. 5213). The next day, the cells were rinsed twice with HBS and dried by keeping the dishes up-side down until the cells showed hardly any damage that could be observed during the subsequent incubation. They were then irradiated with UV light from both the top and bottom of the dishes, at 23 and 2.8 J m⁻², respectively. The irradiated cells were incubated in the growth medium at 37°C for a further 6 days then the cultures were again irradiated with UV light at 1.5 J m⁻² from both sides of the dishes, by the procedure described above After incubation in the growth medium for 2 weeks surviving colonies were scored after formaldehyde fixation and staining with Giemsa. The values shown are the averages of results from five independent experiments b, A204 DNA was digested with restriction enzymes (Takara Shuzo or New England BioLabs); 2 units of enzyme per µg DNA, at 37 °C for 4 h Complete digestion of DNA samples with these enzymes was monitored by adding a small amount of bacterrophage \(\lambda\) DNA to an aliquot of each reaction mixture, and analysing the digestion products by gel electrophoresis. The cleaved DNA samples were precipitated with 70% ethanol, dissolved in a small amount of 10 mM Tris-HCl, 1 mM

EDTA pH 7 2, and transfected as described above.

* The DNA was treated with 1 mg ml⁻¹ pancreatic DNase I (Sigma, type I) in 10 mM Tris-HCl, 5 mM MgCl₂ pH 7.2 at 37 °C for 60 min and then transfected.
† Number of minute colonies detected (see also Fig. 2) in which cells did not survive after colony isolation.

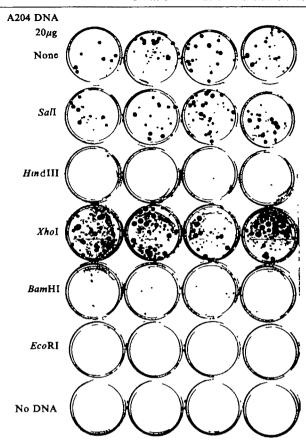


Fig. 2 Petri dishes containing the UV^r colonies detected in the experiments shown in Table 1b. The four dishes in each row were derived from one transfected culture. The restriction enzymes used to digest the transfecting DNA are indicated at the left-hand side.

cleavage sites (see Table 1b and Fig. 2). SalI and XhoI raised the transformation efficiency whereas HindIII, BamHI and EcoRI removed the transforming activity. On the other hand, considerable numbers of minute colonies were detected in the cultures treated with BamHI- and HindIII-digested DNAs; these digested DNAs seemed to raise slightly the UV-resistance of the recipient cell, possibly by abortive transformation. However, we were unable to characterize further the cells in these minute colonies because they did not survive after colony isolation.

The UV-resistant XP20S(SV) cells transformed by both high-molecular weight and restriction endonuclease-cleaved DNA from A204 and MRC-5, showed stable UV phenotypes during passage in culture; we found no revertants that were highly sensitive to UV irradiation.

These results suggest that the DNAs of non-XP human cells contain a gene(s) which confers the UV phenotype on the XP cell, and which carries the BamHI, EcoRI and HindIII sites. SalI and XhoI cleave only outside the functionally important regions of the gene. The gene is not neccessarily the wild-type counterpart of the defective gene in the XP cell, but it could be any gene which can suppress the UV-sensitive phenotype of the XP cell. We are now cloning the DNA sequences carrying the transforming activity using the selection of UV transformants described in this study.

We thank Dr Hiraku Takebe for supplying XP20S(SV) cells. This work was supported by grants-in-aid from the Japanese Ministry of Education, Science and Culture.

Received 6 January, accepted 3 February 1982

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Regulation of DNA ligase activity by poly(ADP-ribose)

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Nuclear ADP-ribosyl transferase (ADPRT)1,2 catalyses the transfer of ADP-ribose from NAD+ to chromatin proteins to form mono-, oligo- and poly(ADP-ribose)-modified chromatin proteins3-7. The enzyme is entirely dependent on DNA for activity⁸ and is activated by nicks in the DNA⁹⁻¹¹. Both radiation and alkylating agents deplete cells of NAD which is used to make (ADP-ribose), (refs 1, 2, 12, 15-17), and this depletion is prevented by inhibitors of ADPRT 13,15,18-25. ADPRT inhibitors also retard DNA strand-rejoining after damage^{1,2,26,27}, and potentiate the lethality of DNA-damaging agents^{1,2,28}. N-methyl-N'-nitro-N-nitrosoguanidine, an alkylating agent, transiently increases intracellular (ADP-ribose)_ over 100-fold¹⁷. These data suggest that (ADP-ribose), blosynthesis is necessary for effective repair of some types of DNA damage1. The question remains of which step in DNA repair requires ADPRT activity. The enzyme inhibitors do not prevent incision events^{1,2,27} nor do they inhibit repair replication² Here we describe experiments which support the hypothesis that ADP-ribosylation activates DNA ligase activity. We suggest that ADPRT has a general function in the control of the breaking and rejoining of DNA.

Mammalian cells possess two forms or species of DNA ligase, which have been chromatographically separated³²⁻³⁹. DNA ligase I seems to be involved mainly in replication, while DNA ligase II may be a repair enzyme^{32,33,37-44}. DNA ligase activity may be extracted from cells with 0.5 M salt and Triton X-100, the enzyme activity being reasonably reproducible. In both control cells and cells treated with the monofunctional alkylating agent dimethyl sulphate, the maximum amount of enzyme activity is extracted by 300 mM KCl. Doubling the salt concentration to 600 mM does not extract any further enzyme activity. The enzyme activity may be estimated by measuring the amount of ³²P-5'-phosphate in nicked DNA that becomes inaccessible and therefore resistant to alkaline phosphatase because the DNA ligase activity has converted the 5'-phosphate -to a 3',5'-phosphodiester. We found that on exposure of mouse leukaemic L1210 cells to dimethyl sulphate, the total DNA ligase enzyme activity increased ~2.3-fold (Table 1).

This increase in enzyme activity was prevented by all classes of ADPRT inhibitor, indicating that it is a consequence of ADPRT activity (Table 1). The specificity of the ADPRT inhibitors is indicated by the inability of their non-inhibitory analogues, aminobenzoic acid and nicotinic acid, to prevent the enzyme stimulation (Table 1). Treatment of cells with ADPRT inhibitors does not inhibit the basal DNA ligase activity or the basal or stimulated ligase activity when present in the ligase assay (data not shown).

Again, the stimulating effect on DNA ligase activity of prior exposure of the cells to the alkylating agent is seen following purification of the enzyme by ammonium sulphate precipitation followed by successive chromatography on phosphocellulose and hydroxylapatite columns (Fig. 1). The total recovered enzyme activity of the DNA ligase peak eluting from the phosphocellulose column increased from 3.05 to 5.42 pmol min⁻¹. Hydroxylapatite chromatography of the DNA ligase peak from the phosphocellulose column shows that ligase II is the enzyme predominantly responsible for the increase in activity (Fig. 1). Whereas the ligase I activity eluting from the hydroxylapatite column increased from 1.90 to 2.40 pmol min⁻¹, that of DNA

ligase II increased from 1.27 to 6.20 pmol min⁻¹, a fivefold increase.

An antiserum has been described by Soderhäll and Lindahl³³ that specifically inhibits DNA ligase I from calf, human, rabbit and mouse tissues but does not affect DNA ligase II from the same sources to a detectable extent. We found that this antiserum, at a 1:8 dilution, inhibited our DNA ligase I enzyme activity (see Fig. 1) by 95% but inhibited ligase II enzyme activity (see Fig. 1) by only 11%, which we attribute to contamination of the ligase II peak by some ligase I material. We conclude that our two ligase enzyme activities are identical to those described by Soderhäll and Lindahl³³.

The fact that we used a high salt concentration means that the increase in ligase II activity is probably not due to differential extraction. This possibility is further excluded by the data in Fig. 1 which show that the specific enzyme activity (units of enzyme activity per unit protein) increases sevenfold on exposure to dimethyl sulphate.

We next investigated whether the blocking effect of these ADPRT inhibitors on dimethyl sulphate-stimulated total enzyme activity was specific for DNA ligase II activity. We treated two sets of cells with 300 μ M dimethyl sulphate, one set being simultaneously exposed to 5 mM 3-aminobenzamide, then extracted and partially purified the DNA ligase activities as described earlier. Again, dimethyl sulphate increased the DNA ligase II activity (Fig. 2a). However, this increase did not occur when 3-aminobenzamide was present (Fig. 2b).

One possible explanation of the above results is that DNA ligase II itself, a closely associated protein or some other

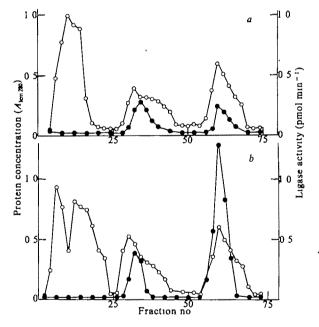


Fig. 1 Increase in DNA ligase II activity after exposure of L1210 cells to dimethyl sulphate. The cells $(100\,\mathrm{ml},\,5\times10^5~\mathrm{cells~ml^{-1}},\,\mathrm{for~each~experiment})$ were incubated for 1 h with or without 300 $\mu\mathrm{M}$ dimethyl sulphate. Salt extracts were prepared as described in Table 1 legend. Ammonium sulphate was added to 75% saturation. The precipitate was collected, redissolved in 15 mM potassium phosphate buffer pH 7.2 and dialysed against the same buffer overnight. A column of phosphocellulose (1.25 \times 7 cm) pre-equilibrated with 15 mM potassium phosphate buffer pH 7.2 was loaded with the dialysed material and washed with 4 ml 15 mM potassium phosphate buffer pH 7.2. Elution with 800 mM NaCl, 15 mM potassium phosphate buffer pH 7.2 continued until no more protein was detected in the cluant. Fractions of 1.0 ml were collected and assayed for DNA ligase activity as described in Table 1 legend. The enzymatically active fractions from the phosphocellulose columns were pooled and loaded directly on to columns of hydroxylapatite $(1.0\times3\,\mathrm{cm})$ pre-equilibrated with 15 mM potassium phosphate buffer pH 7.2. Stepwise elution was carried out with increasing concentrations of potassium phosphate buffer pH 7.2; 1 ml 15 mM, 4.0 ml 50 mM, 5.0 ml 140 mM, 5.0 ml 400 mM. Aliquots (200 µl) were collected and assayed for protein concentration and DNA ligase enzyme activity. O, Protein concentration (A_{280} absorbance units); \bullet , DNA ligase activity (pmol min⁻¹). a, Cells not treated with dimethyl sulphate, b, cells exposed to 300 µM dimethyl sulphate for 1 h

Table 1 Stimulation of DNA ligase activity by an alkylating agent, and its prevention by inhibitors of ADPRT

	Relative DNA ligase activity Exposed to			
Additions		Controls'	dimethyl sulphate	
None	(10)	1 00	2.35	
Benzamide	(2)	1.08	0 81	
3-Ammobenzamide	(10)	0.80	1.08	
5-Methylnicotmamide	(2)	1.10	0.85	
Theophylline	(2)	0.94	. 1.06	
Thymidine	(2)	0.94	0.97	
3-Ammobenzoic acid	(2)	- 0.90	1.82	
Nicotime acid	(2)	1.06	1.82	

Mouse lymphoblastic leukaemia cells (L1210 cells) $(5 \times 10^5 \text{ cells ml}^{-1})$ were treated with $300~\mu M$ dimethyl sulphate for 1 h in the presence or absence of the inhibitors (5 mM) or their analogues (5 mM). DNA ligase activity was then extracted from the cells. In control experiments the cells were exposed to the inhibitors or analogues, but were not treated with dimethyl sulphate. The cells were centrifuged and then washed twice with phosphate-buffered saline. The cell pellet was then resuspended in 1/10 the original volume of extraction buffer at 0 °C. The extraction buffer consisted of 500 mM KCl, 20 mM Tris-HCl pH 7.5, 0.5% (v/v) Triton X-100. The cell pellet in the extraction buffer was sonicated twice in an MSE 150-W sonicator for 15 s each time. The enzyme extract was recovered by centrifugation at maximum speed in an Eppendorf microcentrifuge. The supernatant contained the DNA ligase activity; this supernatant was dialysed overnight against 20 mM Tris-HCl pH 7 5, 1 mM mercaptoethanol. DNA ligase activity was estimated with ³²P-5'-phosphate DNA as substrate, prepared according to ref. 45. The DNA ligase assay measures the rate at which ³²P-5'-phosphate becomes inaccessible to alkaline phosphatase because of DNA ligation. The enzyme assay mixture (200 µl) contained 75 mM Tris-HCl pH 7.5, 15 mM MgCl₂, 200 µM ATP, 2 mM dithiothreitol, 20 µg bovine serum albumin, 20 pmol ³²P-5⁷- phosphoryl DNA (1,000 c.p.m. pmol⁻¹), 100 µg ml⁻¹ protein from the enzyme extract. The assay mixture was incubated at 30 °C for 30 min. The reactions were stopped by the addition of 5 μ mol EDTA and 15 μ l 1.0 M Tris-HCl pH 8.5 at 0 °C. The ³²P-phosphate that had become resistant to alkaline phosphatase was estimated according to the procedure described by Weiss et al.*6. One unit of DNA ligase activity catalyses the conversion of 1 pmol of ³²P-5'-phosphate to phosphatase-resistant material in 1 min in the above conditions. The DNA ligase assay is linear with time at least up to 60 min, regardless of whether the cell extracts have been exposed to dimethyl sulphate. Values in parentheses indicate the number of times each experiment was performed. All enzyme activities are expressed relative to the activity in cells not exposed to dimethyl sulphate, inhibitors or their analogues.

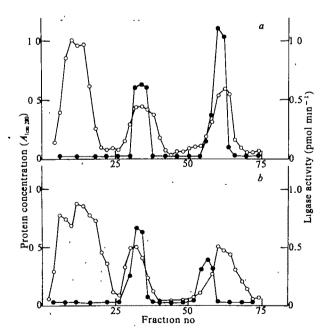


Fig. 2 3-Aminobenzamide prevents the increase in DNA ligase II activity observed after exposure of L1210 cells to dimethyl sulphate. The cells (100 ml, 5×10⁵ cells ml⁻¹, for each experiment) were incubated for 1 h with 300 µM DMS. One set of cells was treated simultaneously with 5 mM 3-ammobenzamide. Salt extracts were prepared as described in Table 1 legend and partial purification of DNA ligases I and II was carried out exactly as described in Fig. 1 legend. O, Protein concentration (A₂₈₀ absorbance units), •, DNA ligase activity (pmol min⁻¹). a, DNA ligase from cells exposed to 300 µM DMS, b, DNA ligase from cells exposed simultaneously to 300 μ M DMS and 5 mM 3-amihobenzamide.

DNA-binding protein is ADP-ribosylated. Preliminary experiments indicate that when permeabilized cells are incubated with ³H-NAD, a specific precursor of (ADP-ribose)_n, a peak of radioactivity co-migrates with the DNA ligase II activity on a hydroxylapatite column.

Thus, we observe that alkylation damage increases DNA ligase activity, predominantly that of DNA ligase II, the presumed repair enzyme. This increase in activity is totally

prevented by inhibiting (ADP-ribose), biosynthesis.

(ADP-ribose), biosynthesis is required for effective DNA excision repair of some types of DNA damage1, while ADPribose is required neither for the incision events^{1,2,28} nor for repair replication³⁰⁻³². We propose that (ADP-ribose), biosynthesis is required for effective DNA excision repair because it is necessary for efficient ligation. The nuclear ADPRT enzyme is stimulated by breaks in the DNA and subsequently stimulates their ligation. However, we suggest that (ADP-ribose), may have a more general role than that of DNA repair, in that it may be sensitive to breaks and regulate subsequent DNA ligation in DNA recombination, sister chromatid exchanges, gene rearrangements, transpositions and cell differentiation which may involve such changes in DNA structure.

This work was supported by the MRC, the SERC and the Cancer Research Campaign. We thank all our laboratory colleagues for constructive help and advice, and Dr S. Soderhäll and T. Lindahl for giving us their specific antiserum against DNA ligase I.

Received 5 October 1981, accepted 27 January 1982

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BOOK REVIEWS

From China with love

J.D. Bjorken

In the spring of 1979 T.D. Lee visited China and within the span of seven weeks delivered a remarkably intense set of lectures on elementary particles and statistical mechanics. The number of lecture hours averaged about three per day, seven days a week, and the size of the audience exceeded 900. Lee reports that "...it was very lively. My vocal chords, however, had problems afterward". Parts of the lecture notes have been published in Chinese and the particle physics portion has evolved into this volume.

It is an especially propitious time for such a course to appear. Because of the recent progress in understanding the nature of the fundamental quark and lepton constituents of matter and of the forces they exert on each other, we now are entering what should be a golden age of pedagogy. A few good texts have appeared and more are on the way. The ideal one is yet to be written. But Lee's lectures, as one might anticipate, set a towering standard of excellence. Within 820 pages of lucid text lie most of the important topics in elementary particle theory.

The central element of the new pedagogy is the belief that the strong and weak forces are described by generalizations of quantum electrodynamics called nonabelian gauge theories. A good understanding of this subject requires mastery of a high level of theoretical formalism. This is not easily found in older texts. Lee's lectures help to bridge the gap between the old and the new, concentrating on the theory of the strong force, called quantum chromodynamics or QCD.

But physical insights have not been sacrificed for formalism. The main physical features of the QCD picture of the interquark force are that it is relatively weak at short distances, and that at large distances it confines quarks — that is, only certain colour-neutral combinations of quarks and their antiparticles can exist in isolation. Lee beautifully elucidates these concepts, albeit with considerable subjectivity on the confinement question. At present, the confinement problem is not closed. There are several approaches extant, such as lattice QCD, instantons and string models. Only one approach, which makes an analogy of the QCD vacuum state with a perfect diaelectric medium, is developed in the book. This reflects Lee's current research interests, and is a characteristic of the lectures. They are selective, not encyclopaedic.

The weak - or, better, electroweak -

Particle Physics and Introduction to Field Theory. By T.D. Lee. Pp.865. ISBN hbk 3-7186-0032-3; ISBN pbk 3-7186-0033-1. (Harwood: 1981.) Hbk \$59.50, Dfl.170; pbk \$19.50, Dfl.60.

force is now also generally believed to be described by a non-abelian gauge theory. Lee's lectures emphasize the older phenomenological foundations, although the prevailing SU(2) & U(1) "standard model" is given a spare but reasonable and up-to-date treatment. Here the computational technology might have been carried further. The physics might have been as well: there is no discussion of the ideas of the grand unification of strong, weak and electromagnetic forces, a topic now inspiring a considerable amount of experimental and theoretical work.

An important link between experiment and the basic gauge-theory concepts is the so-called quark-parton model. This is a simple manifestation of the idea that point-like quark constituents reside within nucleons and other hadrons. The quark-parton model is also an element of the new

pedagogy — one which is simple to apply and of great usefulness. It is a good way to learn a lot of physics easily and quickly. Lee develops it well, with relevant examples worked through in physically transparent ways.

But the core of Lee's lectures lies not in the new pedagogy, but the old. There is a concise introduction to quantum field theory, followed by an extended, masterful and definitive section on symmetry principles and the associated conservation laws. This leads to a full discussion of the neutral kaon system — that glory of wave mechanics and source of so many of our deepest insights into nature.

This volume will serve superbly as a comprehensive course in elementary particle theory at the advanced graduate-school level. It is possible to differ with Lee on questions of balance and of selection of material. But the pages are filled with his unique insights, pedagogical clarity and thoroughness. The book is a treasure.

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A cooperative venture in soil science

Harry Vine

Characterization of Soils in Relation to their Classification and Management for Crop Production: Examples from Some Areas of the Humid Tropics. Edited by D.J. Greenland. Pp.446. ISBN 0-19-854538-X. (Clarendon/Oxford University Press: 1981.) £35, \$74.

This volume is, with a few added contributions, the report of a "bench-mark soils" project which was based at the International Institute for Tropical Agriculture (IITA) at Ibadan, Nigeria. The core of the book, then, consists of detailed studies of 38 Nigerian soil profiles, in which about 20 specialists in soil science took part.

A major aim of the project was "to provide data that might enable existing soil classification systems used within the tropics to be improved"; this related especially to the American system, which was developed through successive Approximations to the massive handbook, Soil Taxonomy, issued by the United States Department of Agriculture in 1975. The profiles are discussed from this point of

view by F.R. Moormann, who points out a number of difficulties in the published scheme for tropical soils, particularly with regard to weatherable sand-size minerals and to the relative importance of the composition of the clay fraction and evidence of its movement in the profile. A fundamental contribution to the characterization of such soils is made by A.J. Herbillon in a review in which he examines both materials and dynamics.

Underlying the work on this range of profiles was the need, in the Farming Systems Programme of IITA, to discern more definitely what measurable properties of humid tropical soils may be relevant to maintenance of fertility in intensified arable cropping, efficient use of fertilizers and avoidance of erosion. In their review chapter, B.T. Kang and R.L. Fox aptly refer to "misconceptions" that these soils "are quite infertile and have management problems which are encountered nowhere else". On phosphate fixation problems, greatly exaggerated in textbooks, good progress is reported by

P.H. Le Mare, who used isotopicallylabelled phosphate in distinguishing strong bonding from exchange-adsorption in laboratory studies of the soils.

Tropical ecosystems have been described as "fragile", particularly in semi-arid regions. D.J. Greenland writes here and elsewhere about "fragile" tropical soils, using the term in a literal sense, implying that particular properties of the soils (not the intensity of the rainfall) were responsible for a tendency to high rates of erosion. Clearly almost all the bench-mark soils, except those of the amphibolite area, can have little stable structure in the topsoil, being sandy loams; but as the sand is medium to coarse the infiltration rate remains fairly high, and run-off and erosion need not be intense. In this connection, some years ago the IITA group devised a system of no-tillage, which involved mulching with plant material, applying herbicides and omitting the yam crop which is important locally. In advocating this they may have been too greatly influenced by the amount of erosion that occurred on experimental plots under rather absurd methods of cultivation; planting on ridges with cross-bars, as on older stations in Nigeria, would have been a better yardstick.

It is also unfortunate that despite the long-term prospects for cattle, with diseases being brought under control, animal husbandry and therefore alternation of arable crops with grass-legume mixtures — which do well in southern Nigeria - were not considered in the Farming Systems Programme. Consequently one of the most interesting features of this book is the inclusion of a long chapter by P.A. Sanchez, "Soil Management in the Oxisol Savannahs and Ultisol Jungles of Tropical South America". Sanchez summarizes much of the very effective work on the characterization of the soils of these regions and also discusses the field experiments at Brasilia and at Yurimaguas and Pucallpa in Peru. Management of cleared land, with lime, fertilizers, legumes and grass, along lines very similar to those appropriate to the south-eastern USA, has been shown to be successful, whereas it was at the stage of clearance that disastrous erosion could occur; on this evidence, Sanchez' recommendation is that clearance should be carried out gradually by slash-and-burn methods.

As A.J. Smyth says in his foreword, Characterization of Soils amply demonstrates the rewards of collaborative research on tropical soils. However readers would be well advised to study it in conjunction with Sanchez' outstanding textbook, Properties and Management of Soils in the Tropics, published by Wiley in 1976.

Harry Vine, who has worked as a soil specialist in Nigeria, was formerly Senior Lecturer in the Department of Geography at the University of Leicester.

The ins and outs of food production

K.L. Blaxter

Biological Efficiency in Agriculture. By C.R.W. Spedding, J.M. Walsingham and A.M. Hoxey. Pp.383. ISBN 0-12-656560-0. (Academic: 1981.) £18.40, \$38.

AGRICULTURE is a very complex undertaking. It involves the use of plants and animals and the deployment of resources of land, labour and capital so to produce food and a range of other products which mankind needs or requires. Using the term "efficient" in the sense "effective". agriculture has obviously been efficient over the centuries for it has resulted in the production of sufficient food to support population growth in the world as a whole and a small but discernible increase in average living standards. Whether agriculture is efficient in the sense of making best use of the resources it deploys is perhaps another question, one to which this study of biological efficiency in agriculture addresses itself.

The major tool employed by the authors in the analysis is that of estimating ratios of the outputs of agricultures per unit time to a number of different inputs, these ratios being termed efficiencies. It is recognized that such single estimates can only apply to specific and highly defined situations, and that the approach can provide little information about the optimization of inputs into agricultural systems. As such, the book is essentially descriptive rather than analytical. It describes crop and animal production systems in many parts of the world in terms of a series of ostensibly simple ratios such as yield per hectare, output per unit of human labour, and yield per unit of fertilizer or water applied and of support energy provided. Annual yields are expressed in terms of dry weight or of energy-yielding constituents edible by man or of protein edible by man. Little emphasis is given to the complexity of the interrelationships between the input terms, that is to response analysis in what must be regarded as a multidimensional system.

A descriptive approach such as that adopted might, nonetheless, have considerable comparative value, so enabling judgements of the relative merits of different crops to be made. The fact that the ratios apply to highly specific contexts of soil, climate, economic and social circumstance, however, makes this impossible. The data assembled in the many tables are interesting as statements about what occurs in farming in different parts of the world. They also have value in predicating a series of other questions in the minds of readers about how to judge the merits of a cropping or stocking system in a particular agricultural setting.

It is wrong to criticize a book because it is about the wrong subject. Nevertheless, a conclusion emerges from this volume which touches on the wider issues, namely that it is important to decrease the input osupport energy into agriculture throughout the world without reducing total food production. Distinctions armade between those uses of support energin terms of fertilizers and agrochemical which augment the efficiency with which incident solar radiation is used to produc food and those in which machines simply substitute for manpower. This is a reaquestion which could well have been developed from a closer analysis of the large amount of descriptive material presented. Perhaps such an analysis is already in the minds of the authors.

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L'animal-machine

J.E.R. Staddon

Quantitative Ethology: The State Space Approach. By David McFarland and Alasdair Houston. Pp.204. ISBN 0-273-08417-8. (Pitman: 1981.) £17.50-\$24-95

Most ethologists accept the idea tha animal behaviour is to be explained in purely mechanistic way. What could be more natural, then, but to turn to engineering, the science of mechanism, for the theoretical analysis of behaviour? So is has seemed to David McFarland and his group at Oxford who for the past 15 year or so have pursued the engineering approach with unequalled thoroughness and sophistication. This book is a summar of their work and the work of others in the same tradition.

The work of the Oxford group has gon through two phases. The initial phase was McFarland's application of linear system analysis to a variety of quantitative motivational problems in which the level of some activity — rate of drinking, or attack or pressing a lever in a Skinner box, of feather fluffing — was measured as a function of a single independent variable usually time. This work resulted in Feedback Mechanisms in Anima Behaviour, published by Academic Pres in 1971.

Simple linear systems analysis represent the state of a system by a single number but this is obviously an arbitrar! restriction. It is much more natural to represent the motivational state of an animal by a point in N-dimensional space. This is termed a state-space analysis by engineers. It also corresponds to a mode o representation commonly used by economists. In the motivational case, each axi

might represent the level of some crucial physiological variable; in the economic case, each axis gives the amount consumed of a commodity. In both instances, the benefit to the individual associated with points in the space is represented by a set of equal-benefit contours. Ouantitative Ethology is largely devoted to descriptions of static and dynamic applications of the state-space approach, the second phase of the Oxford group's programme. The book provides a useful catalogue of methods of analysis and examples of applications. Techniques discussed include the method of Lagrange multipliers (a standard tool for economists), Pontryagin's maximum principle (an approach to dynamic optimization), classical control systems theory, and measurement theory (a discussion of the combination rules describing the collective effect of stimuli and other causal factors on behaviour).

This book differs from McFarland's earlier one in another respect: its emphasis on adaptive function. The formal similarity of the economic and engineering approaches allows the authors to move easily from mechanistic models to optimality models, from an engineering approach where the animal is treated as a control mechanism that seeks to maintain the point representing its internal state within an "acceptable" region, to an economic approach where the organism is assumed to maximize its fitness. In their discussion of decision rules the authors show that they are well aware of the distinction between the limited means animals use to achieve optimal behaviour, and the optimality analyses that define optimal behaviour. Yet the formal similarity between the economic and engineering versions of state-space analysis suggests that further work is necessary to separate the ideal from the means the animal uses to achieve it.

Depending on their definition of "ethology", some will quarrel with the title of the book. If you believe that the distinction between ethology and animal psychology is that the former deals with the ways in which animal species differ from one another, whereas the latter is interested in their similarities, then you will find McFarland and Houston's book closer to psychology than ethology. If you feel that the important questions in ethology are social, or involve ecology neurophysiology, then the book is not for you. On the other hand, if you consider that our best hope for understanding animal behaviour is to begin with the large and potentially quantifiable processes of motivation and steady-state operant behaviour, then Quantitative Ethology presents the clearest exposition to date of the work of the Oxford group on these topics.

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Fruits of Australian botany

David W. Goodall

Flora of Australia. Vol.1, Introduction. Pp.200, ISBN hbk 0-642-06652-3; ISBN pbk 0-642-06653-1. (Australian Government Publishing Service, Canberra: 1981.) Hbk \$A15.50; pbk \$A10.50. The Vegetation of Australia. By Noel C.W. Beadle. Pp.690. ISBN 0-521-24195-2. (Cambridge University Press: 1981.) £50, \$120. Australian Vegetation. Edited by R.H. Groves. Pp.449. ISBN hbk 0-521-23436-0; ISBN pbk 0-521-29950-0 (Cambridge University Press: 1981.) Hbk £27.50, \$64.50; pbk available in Australia only. The Biology of Australian Plants. Edited by J.S. Pate and A.J. McComb. Pp.412. ISBN 0-85564-194-0. (University of Western Australia Press: 1981.) \$A25.

No flora of the whole of Australia has been compiled since George Bentham's Flora Australiansis, published from Kew between 1863 and 1878. It is still a standard reference work, despite the enormous amount of taxonomic research undertaken during the intervening century (twice as many species of higher plants are now known in Australia as were recorded by Bentham). State and regional floras have been written but, for important parts of the continent, there has been nothing to replace Bentham — out of date as it is.

Accordingly, it was a major event for Australian botany when, after 20 years' gestation, arrangements were finally completed in 1979 for the publication of a new flora of Australia, of which the first volume is reviewed here. It is planned that Flora of Australia will cover the vascular plants in 48 volumes, with an unspecified number of subsequent volumes to deal with the lower plants; attention is at present concentrated on the flowering plants, and practically every angiosperm taxonomist working in Australia will be involved in one or other of the volumes.

The present book is explicitly an introduction to the series. The main elements are an excellent chapter by B.A. Barlow on the origin and evolution of the vascular flora; an outline of the system of classification used, in which A. Kanio compares the recent systems of Takhtajan, Dahlgren, Thorne and Cronquist with more traditional ones; and a key to angiosperm families by H.T. Clifford. The last is based primarily on experience in Queensland, and comments are invited as to how satisfactory it proves on the continental scale. Certainly, this section of the volume is likely to be in frequent use by nonspecialists.

A flora is judged, however, not by its introductory material but by the quality of the species descriptions and keys. I have had the opportunity of seeing proofs of another volume in the series, and it is clear that high standards are being set and achieved. Descriptions are clear, including

notes on ecology and geographical distribution (numerous maps are included). The original description and type specimen are identified, and some representative specimens in Australian herbaria are listed.

It is clear that this series will be in constant demand and use wherever serious taxonomic work is in progress. It will be a necessity for all botanical libraries in Australia, and all taxonomic libraries throughout the world.

Two of the other books covered in this review, those of Professor Beadle and Dr Groves, have much in common; both are concerned with vegetation types, have very similar titles and (surprisingly) come from the same publisher. The first, however, is almost entirely one man's work, the second has separate authors for each chapter. Both, but particularly the first, are well illustrated with numerous photographs. Recurring themes in the two volumes are the importance of nutrient status -Australia is a very old landmass, much of which is deficient in various nutrients, particularly phosphorus - and fire as a regular component of the environment to which Australian vegetation has become adapted.

Professor Beadle's compendious volume is the Australian contribution to the series Vegetationsmonographien der einzelnen Grossraume. After preliminary chapters on climate, soils and the flora, including its history and origins (cf. the chapter by Barlow in Flora of Australia), the various plant communities recognized are described seriatim. The communities are distinguished, named and described on the basis of the dominant species in the over-storey. This makes for numerous units ("associations" or "alliances") and for fragmentation of vegetational continua, in which the same under-storeys may continue under various canopies, and even the dominant species may almost be vicariants, replacing one another with little influence on the rest of the vegetation. Particularly is this so in the vegetation dominated by Eucalyptus spp., the description of which occupies one-quarter

The Banksias

Academic Press have recently published the first volume of a three-volume work, *The Banksias*, with paintings by Celia Rosser and text by Alex George.

The project was initiated with the aim of creating a work of both art and taxonomic scholarship. When complete, in 1988, the series will contain illustrations and detailed descriptions of all 72 species of the *Banksia* genus.

Each of the three volumes will be available only in a numbered, limited edition of 720 copies. Price of Vol.1 is £965, \$1,965. The less pecunious may arrange to see the book at the Linnean Society, Piccadilly, London, or at Academic Press offices in London, New York or Sydney

of the book, in accordance with their importance in the continent. The frequent references to ecotones underline the inadequacy of a treatment based on discrete communities defined by dominants.

The approach adopted implies that the description of a vegetation type largely consists of an account of the geographical distribution and habitat preferences of the dominant species, with a list of the species accompanying it in the under-storey. There are no association tables, and very little on the quantitative make-up of the vegetation. The treatment is mainly descriptive; vegetational dynamics are mentioned in some places, but in general the vegetation is treated as static.

The volume edited by Dr Groves is much less ambitious than that of Professor Beadle, and contains far less floristic detail. Although here, too, there is a tendency to write in terms of dominantdefined communities (as has been the tradition in Australia), some of the writers point out the drawbacks of this approach, and consider alternatives: there is in any case no attempt to treat each community separately. In consequence, the book is far more readable than Professor Beadle's. The different vegetation types are very adequately covered, but in broad groups rather than individually. Although there are inevitable differences in the treatments accorded their subject-matter by the different authors (some chapters are mainly descriptive, others have substantial quantitative components, including estimates of productivity; others again devote attention to analytical and dynamic aspects), the editor has had a good deal of success in achieving uniformity and balance.

These two books will fulfil different functions. Professor Beadle's will serve as a constant reference source on the variety, geographical distribution and floristic make-up of Australian vegetation. Dr Groves's, on the other hand, could well be used as a textbook in advanced courses, particularly where a purely descriptive approach is considered unsatisfactory.

The two volumes share some faults. Neither deals with ruderal or segetal communities, or with the sown and lightly managed grasslands which occupy such large areas in the more densely populated parts of the continent. Little attention is paid to non-vascular components of the vegetation. And, surprisingly, little notice is given to the role of domestic livestock in causing changes and determining the current pattern of Australian vegetation.

The fourth volume reviewed consists of papers presented at a symposium in Perth in 1979. The first four contributions cover plant responses to four types of stress important in Australia — lack of water; low nutrient supply; fire; and salinity. At that point, however, planning of the subject-matter becomes less noticeable, and the topics seem simply to reflect the research interests of participants. This does

not, of course, prevent individual papers being of considerable interest; one may mention, for instance, that by D.E. Gaff on "resurrection plants" — those, such as Borya nitida, which can suffer complete desiccation yet resume growth when remoistened (surprisingly, Dr Gaff does not mention lichens and arid-zone mosses, "resurrection plants" par excellence); and

that by A.J. McComb *et al.* on seagrasses. The general standard is higher than that of many symposium volumes, and the book should certainly find a deserved place in botanical libraries.

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Volcanology: last of the common ground

P. W. Francis

Tephra Studies. Edited by S. Self and R.S.J. Sparks. Pp.481. ISBN 90-277-1327-8. (Reidel: 1981.) DM125, \$54.50.

ICELAND is rather an appropriate venue for international meetings, and especially a NATO-sponsored one, since its position astride the mid-Atlantic ridge makes it mutually accessible for scientists from both North America and Europe. This volume is the outcome of an uncommonly successful NATO Advanced Study Institute held at Laugarvatn, Iceland, in June 1980, Several factors contributed to the success of the meeting: the participants lived and worked together in an isolated hotel, distracted solely by spectacular views of the volcano Hekla on the opposite side of Lake Laugarvatn; the exorbitant cost of liquor in Iceland ensured that most of them were able to arrive at the morning sessions fresh and attentive; and the steamy intimacy of geothermally heated sauna baths meant that ordinary barriers of communication were soon broken down. The constructive discussions that flourished in this environment are reflected in many of the contributions to the volume that resulted.

Tephra is the collective noun used to describe pyroclastic rocks. The book is something of a milestone in volcanology because it is the first to be devoted exclusively to this long neglected but increasingly important subject, which impinges on disciplines as diverse as archaeology, climatology and petrology. It is clearly a volume that libraries and tephra specialists should have on their shelves, but it may be less useful to other classes of reader, precisely because it does include such an extremely broad range of topics someone interested in marine studies, for example, would derive little benefit from sections on the use of tephra in dating archaeological sites.

Tephra studies are relatively modern. The term was first coined by the Icelandic doyen of the subject, Sigurdur Thorarinsson, to whom the book is most fittingly dedicated. Thorarinsson's introductory chapter is a model of lucid and stimulating technical writing. It is difficult to forget his account of the early Icelandic worker, who, in order to determine the limits of a tephra fall, was accustomed to pick up a pellet of sheep

droppings and take a bite at it. When he could no longer feel grains of fine ash between his teeth, he knew he was outside the zone of tephra falls. While the other 30 or so contributions do not all maintain the same high standard, and are more conventionally turgid, they are generally informative and well presented. Particularly noteworthy are the accounts by Walker of remarkably violent eruptions in New Zealand; the first serious analysis of the effects of heavy ash falls on buildings and other structures by Blong; and the startling suggestion by Steen-McIntyre that archaeological remains at a site in central Mexico, associated with tephra dated by her using the tephra hydration technique, may indicate the arrival of early man in the New World some 150,000 years before the generally accepted date.

Like many other conference proceedings, the book has been produced in camera-ready copy format. The quality of the text is perfectly adequate, but a few of the diagrams have suffered somewhat in the reproduction process from excessive reduction. A few proof-reading errors occur, but much more irritating is the editors' decision not to include the titles of papers in references. Although the space saved by their omission must be significant, so too is the inconvenience caused to readers. Other editors should be urged to reconsider their policy in this respect.

Although the book is an important contribution to the literature of volcanology, it seems unlikely that such a comprehensive study will be repeated soon! There is such a gulf between, for example, the interests and backgrounds of volcanologists studying the mechanisms of emplacement of pyroclastic flows and stratigraphers working on the use of ash layers as marker horizons in the Quaternary, that there is unlikely to be much common ground between them, and future more detailed studies will reduce this still further, until the only common factor will be the pyroclastic origin of the rocks concerned. We must welcome the publication of this book, then, as merely the first of many more specialized ones in the headlong advance of tephra studies.

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nature

25 March 1982

Supporting international safeguards

The US State Department must now persuade Congress to back the international safeguards system — and then worry about what will happen a decade hence.

Not before time, the United States is edging in the right direction in its policy on the proliferation of nuclear weapons. Almost two years have gone since President Reagan, then merely a candidate for his office, began promising that he would repeal President Carter's Nuclear Non-Proliferation Act, passed by Congress in 1978. Now, it seems, the Administration is planning to do what it can, within the framework of the act, to remove some of the sillier restrictions on the supply of nuclear equipment from the United States made necessary by this unfortunate legislation (see page 279). But there is still no sign of when it plans to carry the fight to Congress, seeking at least to amend the Carter act. Mr James Malone's departure from the State Department (Nature, 18 March) may be a sign of impatience, but mere irritation is not a substitute for action. Part of the trouble seems to have been that the Reagan Administration, while staunchly opposed to the Carter act on the doctrinal grounds that it needlessly restricted the freedom of American suppliers to sell nuclear equipment overseas, has not fully understood why the Carter act is self-defeating. Now, to judge from the good sense of what the State Department was saying last week about the problems of nuclear proliferation, enlightenment is at hand.

How can a piece of legislation with such laudable objectives have the opposite effect, of assisting the proliferation of nuclear weapons? The explanation, now familiar, is not all that complicated. Between 1965 and 1970, the United States took the lead in persuading non-nuclear powers to sign the Non-Proliferation Treaty, then offered as the best way of controlling the spread of nuclear weapons. Persuasion was necessary because, to many non-nuclear powers, it was not self-evident that a treaty that did not restrict the right of nuclear powers to keep on making nuclear weapons could serve the stated purpose. Against the odds, however, persuasion worked. More than a hundred non-nuclear powers have ratified the treaty and have agreed to put up with repeated and unavoidably irksome visits by inspectors from the International Atomic Energy Agency.

The Nuclear Non-Proliferation Act, especially as interpreted by the Carter Administration, was a shock to this system because it signalled that in the opinion of the United States, even strict compliance with the terms of the treaty would be insufficient. The act was also a unilateral denial of the promise in the treaty that the nuclear powers would help with the free flow of nuclear technology. And by implicitly asserting that compliance with the international safeguards system would not be taken as an assurance that a signatory of the treaty was not making nuclear explosives on the sly, the act undermined the credibility of the safeguards system as a whole. No doubt the consequences would have been more serious if the civil nuclear industry had not been in such doldrums in the past few years.

The new enlightenment that has overtaken the United States Administration, well put by Mr Eugene Rostow last week, is that technical devices such as the safeguards system operated from Vienna are necessary but not sufficient means for controlling the spread of nuclear weapons. Now, as since the invention of nuclear explosives, the decisive determinants of the spread of nuclear weapons are likely to be political — that is, governments' perceptions of the international dangers that confront them and their calculations, never straightforward, of whether nuclear weapons would help or, by provoking imitation, hinder. So

governments such as that of the United States seeking to limit the spread of weapons can hope to accomplish more by diplomacy than by tinkering with the Vienna safeguards. After all, the non-nuclear powers best placed to make nuclear weapons for themselves — India, Israel, Pakıstan and South Africa, for example — are not even signatories of the treaty. In this sense, the preoccupation of the United States Congress (and of two unreflective members of the Nuclear Regulatory Commission) with the supposed loopholes in the safeguards system are strictly speaking irrelevant, and should be recognized as such. Luckily, the Administration seems to have accepted the point. Its task now is to persuade those who continue to grumble that even though it is not feasible to make the safeguards system strictly watertight, that does not imply either that the system is without value or that it should be scrapped.

No safeguards system can be perfect. The Vienna system now in force cannot, for example, guarantee that some signatory of the Non-Proliferation Treaty has not built clandestine nuclear plants in some remote part of its territory. (Surveillance satellites, however, can.) Nor can it prevent a government that has privately decided to make nuclear explosives from taking advantage of the intervals between physical inspections secretly to divert fissile materials to some hidden bomb-making plant. It is, however, unthinkable that any government would invite the trouble that would follow when it could achieve the same objectives by withdrawing from the treaty at three months' notice. Then even the best-designed monitoring instruments can break down, and the most alert inspectors be misled. The pursuit of perfection is therefore pointless. So long as the chance of the detection of violations of the rules is substantial, the international community has a reasonable assurance that signatories of the Non-Proliferation Treaty are not breaking the rules.

None of this implies that the safeguards system needs no attention. But its most serious weaknesses are not technical but administrative and financial. The new director-general of the international agency, Dr Hans Blix, has been stressing in the past few weeks that the technical qualifications of his small but rapidly growing staff of inspectors should be improved. But where will these people come from? As things are (or were at the end of 1981), 130 qualified inspectors were required to inspect a total of 850 nuclear installations, but were able to carry out only half as many inspections as they should have done. What will happen when the number of nuclear installations under safeguards has grown by an order of magnitude, and when inspections will have to be more frequent because the quantities of fissile material involved will be much larger? On the face of things, the international inspectorate will need several thousand skilled people. Is it sensible to think that they will materialize, or that their work could be organized effectively?

The danger that at some point in the future the safeguards system will collapse under its own weight is thus perhaps the most serious threat to the integrity of the whole system. What can be done to head it off? In the short term, technology has much to offer. Automatic devices for monitoring what happens in nuclear plants have been installed within the safeguards system, and will no doubt be improved (see page 279). Devices that could sample data from a distance would be potentially invaluable. There is also some reason to believe that inspection could be simplified if those

0028-0836/82/120277-02501 00 € 1982 Macmillan Journals Ltd

who operate reactors, for example, would agree that a continuous and incorruptible record should be kept of, say, neutron flux or some other physical quantity sensitive to the movement of nuclear material within them. The safeguards system now in use, devised merely to account for quantities of fissile material in the input to and the output from a reactor, does not require the collection of such data — which would in some places be regarded as an infringement of national sovereignty. It is not too soon to plan for a return of this provision at the next meeting of the signatories of the treaty three years from now.

Further ahead, there is a strong case for planning for a much more radical simplification of the safeguards system. Even as things are, governments with nuclear installations on their territory will, if they are prudent, take steps to make sure that fissile material is not spirited away. These domestic interests thus coincide with those of the international safeguards inspectorate, but the work is duplicated. The ideal, then, would be that self-policing should be done in such a way that it could be unambiguously and internationally verified. (Some concessions to this notion are already made within the Euratom system, which has its own set of safeguards.) The sooner this goal is recognized, the more likely it is to be attained. And that, in the long run, will be the best assurance that this important instrument in the non-proliferation system remains intact.

Problem not for now

Is it too soon to be worrying about who should operate the shuttle system?

If this week's space shuttle, diverted by the aftermath of heavy rain from California to New Mexico, returns successfully, the chance that it will become a white elephant will substantially have diminished. The first two flights have shown that the machine will function as intended. This time there should be information bearing on its potential usefulness. But it will be a long time before anybody will known that the shuttle will do the job for which it has been built — to put large satellites cheaply into orbit. The immediate need is to reduce the intervals between successive flights of the one machine in service — an interval artificially lengthened by the need to return it from its landing site to its launching pad in Florida but also by the need to replace whatever ceramic tiles have fallen off in flight. The plan is that the next flight but one should take off from and land in Florida, but only when there are four machines in service will it be possible to tell whether the turn-round time is really as short as planned.

So why is the United States government already in a tizzy trying to decide within what legal framework the shuttle should be operated in the closing years of this decade? It is not even as if the problem is all that novel. The development of telecommunications satellites in the 1960s is an obvious precedent. Then, as with the shuttle, there were three kinds of customers in sight — the US Department of Defense, potential users of communications satellites in the United States and customers from elsewhere, principally the international consortium of communications authorities called Intelsat. The largely successful solution was to leave the launching of military satellites to the Pentagon and to set up the corporation called Comsat as an organization independent of the government for launching and managing communications satellites.

Operating the shuttle commercially will be more complicated only in two respects. It may turn out to be uneconomic to have separate spacecraft for civil and military launchings, while, for a time at least, whoever owns the first four spacecraft will enjoy a monopoly of some kind. But there is no reason why a corporation along the lines of Comsat should not occasionally work for the military, and no reason why the temptation to make outrageous profits should not be restrained by a modicum of regulation. These difficulties will be clarified only several years from now. In the meantime, it might be thought, the US government has more urgent problems crying out for its attention.

Europe in the doldrums

Could science and technology help the EEC to a second twenty-five years?

What is to become of the European Community, which will be celebrating its twenty-fifth anniversary this week? On recent form, member governments will use what energy they can spare for the Community on now-familiar disputes about the prices that farmers should be paid for various foodstuffs or the shares to which they are entitled from this and that central fund. In their defence, governments will say that in the middle of a recession and with more than 10 million people unemployed in Europe, this cannot be the time for pausing in the pursuit of self-interest. They will also rightly say that a great deal has been accomplished in a quarter of a century. There is a customs union which works reasonably well. The Community has been enlarged (from six to ten, but Greece is shaky). And there is a sense that Europe is culturally more of a piece than it was. The snag is that the benefits are intangible, so that it is the public quarrels that stick in people's minds.

That things should have come to such a pass is easily understood. The treaty signed in Rome on 25 March 1957 was necessarily a blend of idealism and practical politics. The earlier collapse of the plan to set up a European Defence Force had warned the negotiators that even the tiniest infringement of national sovereignty would have to be made explicit and agreed in advance. Although there has recently been some talk of concerted action on defence the European Community is unlikely to be chosen as the vehicle.

The tentativeness of the past quarter of a century means that even now the Community does not enjoy economic cohesion. While private companies are required to compete with each other freely, governments can and do bias their purchases in favour of their own national suppliers, thus denying all members of the Community the benefits of scale and of an economic division of labour in some of the most important fields of technology. There was a minor sensation when British Telecom ordered new exchange equipment from a non-British corporation, but nobody appears seriously to have suggested that the British Central Electricity Generating Board should order the pressurized water reactor it wants to build from say Framatome (see page 299). Yet the European Commission in Brussels is wringing its hands over what used to be called the "technology gap" and seeking some way of strengthening the industries that its members have themselves weakened by their purchasing policies. Would it not be more productive to work out some set of inducements for persuading the member governments that they must give up their technological chauvinism?

Much the same question should soon be asked about the support of Community governments for research. Over the past quarter of a century spending by the European centre on research has if anything been set back. At the outset there was Euratom, but now there is merely the Joint European Torus and a miscellaneous programme of research at the old Euratom laboratory in Italy. Otherwise, governments deal independently with their spending on research, making separate decisions about their membership of international organizations or their spending in their domestic laboratories.

Up to a point, all this is justifiable. Governments responsible for universities also have to equip them for carrying out research, but even here there is scope for planning on a European basis complementarity between the centres of excellence that different governments support. Elsewhere, it is shocking that so little has been done to coordinate research on problems or fields of common interest. Should not, for example, something be done to concert the very considerable efforts in agricultural research, not so as to save money but in the hope of becoming more effective? The stock answer, that coordination works only inefficiently, is another way of saying that Europe is better balkanized. It would be better to devise machinery that made efficient collaboration possible.

US backs nuclear safeguards

Rostow hints at new policy on plutonium

Washington

The Reagan Administration rallied behind the International Atomic Energy Agency (IAEA) last week, claiming that a strong international safeguards system was essential for the development of nuclear commerce. It argued that weaknesses in the safeguards system should be remedied by greater support for IAEA programmes, not by changing their basic objectives.

The Administration's views were given in testimony to two subcommittees of the House of Representatives' Foreign Affairs Committee. One issue was the adequacy of the Administration's proposed contribution to IAEA in the 1983 budget. Although the increase over the 1982 contribution is larger than that for any other international organization, it will still not be enough to keep up with inflation — a source of concern to some State Department officials faced with rapidly growing demands for IAEA inspection procedures.

The hearing was also an opportunity for the State Department to rebut some recent criticisms of IAEA, in particular complaints about its admission last year (in connection with a reactor in Pakistan) that it cannot always assure member countries that nuclear materials are not diverted from peaceful to military purposes.

Such criticisms, State Department officials argued last week, are not only misdirected but also potentially harmful, tending to undermine the credibility of IAEA. Mr Richard T. Kennedy, Under-Secretary of State for Management and head of the delegation to IAEA, angrily rejected charges by Congressman Richard Ottinger that the agency had been involved in a "cover-up" by not making public its concern about the possibility of the diversion of nuclear material in countries such as Iraq and Pakistan.

Mr Kennedy was accompanied at the witness table by Ambassador Richard Kirk, deputy US representative to IAEA, and Dr Eugene Rostow, head of the Arms Control and Disarmament Agency. Each spoke strongly on the theme that the agency requires as much support as possible from the industrialized nations, and that the adequacy of safeguards should not be considered in isolation, but merely as one element in the control of nuclear proliferation.

Thus Mr Rostow told the subcommittees that halting the spread of nuclear explosives was "inconceivable" without IAEA safeguards, but added that they did not prevent diversion since, for example,

they did not permit searches for clandestine materials or facilities. "In my view, it is just as wrong to overestimate the importance of safeguards in nuclear commerce as it is to denigrate the system for not accomplishing objectives for which it was not designed," Mr Rostow said.

The State Department's consensus on IAEA, however, was not shared by all members of the Nuclear Regulatory Commission (NRC). Under the terms of the Nuclear Non-Proliferation Act of 1978, the commission is responsible for checking that safeguards are applied to any foreign nuclear installation to which nuclear materials are being exported from the United States.

Mr Peter Bradford, on his last day as one of the five members of NRC, was outspoken about the difficulties experienced by both NRC and congressional committees in obtaining data by which to assess the effectiveness of the safeguards. At one point, he accused the State Department of unnecessarily censoring NRC's reply to questions submitted by Congressman Richard Ottinger.

Mr Kennedy refuted the charge of censorship, pointing out that the information was being withheld at the request of the Central Intelligence Agency—which has since offered to brief Mr Ottinger on the subjects that he had inquired about. Both he and Dr Rostow, however, declined to say whether the State Department has evidence of the diversion of nuclear materials.

Both Mr Bradford and a second NRC

commissioner, Mr Victor Gilinsky, expressed reservations about the adequacy of IAEA inspection procedures for warning about the diversion of weaponsgrade plutonium from reprocessing or enrichment facilities — concerns which led the Carter Administration to attempt to dissuade other countries from adopting such technologies.

Dr Rostow in reply criticized the previous Administration's approach, suggesting that attempts to impose unilateral controls could backfire by encouraging the spread of reprocessing while making less likely the agreements on a common policy with other nuclear suppliers. The Administration's policy is soon to be defined in a new executive order which Mr Reagan is to sign; Dr Rostow said it was important to acknowledge that civil reprocessing in the stable industrial democracies did not in themselves present a proliferation risk.

On the IAEA safeguards, Dr Rostow urged that member states should provide the international safeguards system with the resources needed. Mr Kirk, however, told the subcommittees that as the number of installations under IAEA safeguards had risen from 560 in 1977 to 850 in 1981, even though the IAEA budget allowed much faster growth for safeguards than in other activities, its expansion of the safeguards system had caused "a resources pinch, growing pains in IAEA's administrative structure, and a lag in IAEA safeguards coverage".

David Dickson

Nuclear monitoring by telephone

Washington

A scheme for collecting nuclear safeguards information by means of telephone lines is to be discussed at a meeting planned for Vienna in June this year. The system, called the Remote Continual Verification programme (or "RECOVER"), which was given a systematic trial in the autumn of 1980, has grown out of the technical proposals for the remote verification of arms control agreements in the draft of the Comprehensive Test Ban Agreement, uncompleted since the end of 1980.

In evidence to the House of Representatives last week, Dr Eugene Rostow said that on the basis of a cost-benefit study carried out at Brookhaven National Laboratory, the Administration was now hoping that the system could be put into service soon, and that it might even be valuable in the verification of treaties (yet to be negotiated) on chemical and biological weapons.

In the nuclear context, the new system is a means of making sure that automatic monitoring equipment does not break down between visits by inspectors from the International Atomic Energy Agency (IAEA). This is done by the repeated but irregular interrogation of monitoring equipment by means of signals transmitted on the international trunk telephone system. So that the authorities responsible for safeguarded nuclear installations cannot corrupt the signals received and sent, signals are encoded by means of an unbreakable code of the type developed for use in what is now called public-key crytography.

The Brookhaven study has apparently shown that the new monitoring system is potentially most valuable in nuclear installations such as reactors which can be refuelled on load and in fast critical assemblies, to which frequent visits from inspectors are at present required. One critical assembly in Japan, containing a fixed quantity of 300 kg of plutonium and 200 kg of enriched uranium, has on present criteria to be inspected every week or two.

The new monitoring system, by reducing the frequency of inspections, would save an estimated \$200,000 a year at that installation alone. The system is, however, unlikely to be used at separation plants (where inspectors are virtually permanently in residence) or in light-water reactors (where redundant monitoring equipment is probably cheaper).

In Japan, the system is also being advocated as a means of safeguarding the sea transport of fissile material, providing a means of making sure that cargoes are not illicitly diverted on the high seas. In such an application, communication would be by means of Earth satellites of the kinds being developed for marine navigation. The effective use of the system for the inspection of nuclear installations on land is thought to require the development of a network of IAEA field stations (of which there are at present only two, in Toronto and Tokyo) and the existence of an efficient trunk telephone system (found wanting in Bulgaria during the 1980 trial of the system).

The application of the system to the monitoring of agreements on chemical and biological weapons presupposes the design and installation of effective automatic monitoring equipment at plants covered by an agreement.

Soviet nuclear power

Signs of caution

The Soviet nuclear energy programme is running into difficulties. In spite of the high priority given to power station construction, last year's targets were not met. And although no specific reference has been made to the need for greater standards of safety, Pavel Falaleev, first deputy Minister of Energy and Electrification, has said that the safety requirements of power stations were being tightened despite the radiation around them being "considerably below the permissible limit and practically no different from natural levels ". In a Soviet context such a remark is sufficient to indicate considerable high-level rethinking.

The discussion of the nuclear programme began about six weeks ago, with a meeting of the Central Committee of the Communist Party to review shortcomings, where the emphasis was on the logistics of construction although inadequacies in the design sector were noted.

Three weeks later, the theme was taken up in a leading article in *Pravda*. This at first followed the line of the central committee meeting, noting that production-line nuclear power units had already been developed, and that supply problems could be dealt with by economic sanctions to penalize those who held up the plan. *Pravda* then went on, however, to suggest serious deficiencies in the design sector.

Here, it was claimed, the Ministry of Energy and Electrification had failed to exercise its supervisory duties. Changes in materials and specifications had been made without either proper justification or the

Sticky problem over Iraq fuel supplies

Two French nuclear physicists, backed by three prestigious members of the Académie des Sciences, have warned President Mitterrand in a report that there would be no easy way to stop Iraq making bomb-grade plutonium—if France rebuilt the Osirak reactor destroyed in a bombing raid by Israel nearly a year ago.

This is the second report condemning the reactor sale to be prepared by the two physicists and to be sent — unsolicited — to the president who is said to be sympathetic to the arguments but to be short of apolitical technical advice.

What stung the physicists to produce a second report were widely-reported claims that "caramel"—a low (7 per cent) enriched uranium oxide fuel—was the answer to the problem. If caramel were sold to Iraq to fuel the reactor, in place of the 95 per cent enriched uranium for which it was originally designed, the claims went, Iraq would have no quick route to the atomic bomb.

However, this misses the point, the new report stresses. The caramel fuel would still produce the same neutron intensity in the large pool around the reactor, where test materials are placed to investigate their reaction to neutron bombardment. If these test materials were replaced by depleted — or natural — uranium (of which Iraq is believed to have supplies) plutonium would be produced in the uranium, and could be extracted chemically.

The only advantage of caramel is that it cannot be used directly to make bombs, whereas 95 per cent enriched uranium is more easily converted. But, the reports point out, four years ago Giscard d'Estaing set limits on deliveries of enriched fuel to Iraq. Fuel would be sent in single reactor-loads of 13 kg, each of which would be loaded into the reactor under supervision and promptly irradiated making diversion technically very awkward. From that time, the diversion of the fuel itself ceased to be a problem — so the use of caramel solved nothing.

The production of plutonium by neutron bombardment, however, could amount to 3.3-8 kilogramme per year (to quote the assessments fo both the Commissariat à l'Energie Atomique and the International Atomic Energy Agency). The amount of plutonium required for a bomb is generally taken to be 6 kg, although under certain circumstances 1-2 kg could be enough. Thus, say the two reports. Iraq could have the capability (in plutonium, at least) of producing a bomb within six months of beginning irradiation, whatever fuel the reactor was loaded with. IAEA inspection, however, might interfere considerably with this rate of production.

France has said it will help rebuild Osirak, provided Iraq guarantees that the reactor will be used entirely for peaceful purposes. The new report may make this politically more difficult for the president.

Robert Walgate

apparatus for selecting the "most progressive" solution to engineering problems. In particular, a proposal put forward by the *Atomenergostroiproekt* design trust, for a change in the structure of the "protective shells" of power stations, was turned down without being studied by the necessary multi-disciplinary panel of experts. The scientific council of the ministry, *Pravda* said, must bear greater responsibility for such decisions.

This reference to protective shells is significant. If, as seems likely, this refers to the concrete containment vessel common in Western power stations, it reflects a change in Soviet policy. Until recently, such vessels were dismissed by Soviet designers as unnecessary, and a capitalist ploy to raise construction costs. Demands from the Finns and Hungarians led them to introduce containment vessels into reactors designed for export. The remark in Pravda suggests that they may now have been introduced into reactors for home use whereas previously it was thought sufficient to surround reactors on the outskirts of major cities with a kilometre or two of parkland or playing fields.

Vera Rich

Intelligence testing

Soviet inequality

The Russians have finally "come out" on the subject of intelligence and other objective tests of performance and personality. The January/February issue of Voprosy Psikhologu (Problems of Psychology) contains three papers endorsing such tests in principle.

The papers are printed prominently at the front of the journal, preceded only by a major statement on future psychology services policy, based on the decisions of the November plenum of the Soviet Union's ruling communist party. The journal quotes President Brezhnev himself in support of a call to concentrate on "scientifically-based solutions to the problems of the nation's education in the service of the scientific and technological revolution".

This is a startling but unequivocal volteface by the Soviet authorities, who have until now (in public at least) regarded "testology" as an instrument of class warfare in the hands of the ruling capitalist élite. Testing was even made illegal in Russia in 1936 by an edict that may now have to be hastily repealed.

One of the most extraordinary admissions in the first paper, by K.M. Gurevich, is that there are still significant class divisions inside Soviet society, as well as national cultural differences within the empire, which test content must take into account.

Western workers in the field of intelligence testing may also prick up their ears at the news that foreign tests are already in use (presumably in the enforced absence of nationally-devised and standardized ones). In fact, it was the use of such tests which originally brought testing into disrepute in the Soviet Union. Gurevich advises: "Above all, when embarking on a study with one of these (foreign) tests, it is vital to decide what contingencies it makes sense to employ in order to make it an appropriate instrument for carrying out an investigation under conditions very different from those under which it was created". A citation at the end of his paper shows that Gurevich recently published a book on the subject (Psychological Diagnostics, Problems and Research, Moscow, 1981).

In the second paper, V.I. Slobodchikov makes an interesting distinction between "suitable" uses of test procedures — that is, they may be used for discrimination and selection but not for "controlling development or in remedial education".

At the opening of the third paper, by Yu. Z. Gil'buch, the British intelligence tester Professor H.J. Eysenck is accused of epitomizing the "mechanistic" attitude to testing. This does not deter Gil'buch, head of a Kiev laboratory, with more than twelve



years of published work on the subject, from concluding his piece thus:

"The fundamental question at the root of any discussion about content validity in intelligence tests is this: what underlies individual differences in the degree of mastery of mental operations as exemplified in any particular culture (as represented in tests)? Is it mostly factors of biological inheritance, innate gifts, or is it (alongside and in interaction with these) the conditions of education and upbringing, which in various families and various schools are unavoidably present in greater or lesser variety?."

Soviet test experts are frantically trying to

devise tests which allow for different norms within classes and national cultures, but which will also serve the practical purpose for which the tests have been revived — namely, the more efficient selection of personnel to man their scientific and technical revolution.

The present aim of Soviet psychologists seems to be to devise tests which tap the purest, least culture-bound workings of the brain, such as solving abstract puzzles, chess problems and devising imaginary games.

Just how important this is to the Soviet Union is summed up by Gurevich thus: "In our country at present the growth of psychological diagnosis has become one of the most vital supports of theoretical and applied studies in the field of education, farming and management. In pre-school education, high school and professional training, methods of psychological diagnosis must be used to assess levels of psychological development: this allows for the inevitability of change within the very process of education, and provides a broad base from which it can advance to rationalization and improvement."

Elizabeth Roberts

Biological warfare

Soviet use

Washington

The Reagan Administration claims that it now has clear and scientifically verified data that the Soviet Union is providing toxic agents for military use by its allies in Laos and Kampuchea, and has circumstantial evidence that the Soviet Union is itself using such weapons in Afghanistan.

The evidence is contained in a report presented to Congress by Secretary of State Alexander Haig last Monday, providing a detailed analysis of eyewitness reports, chemical analysis of samples and information from other sources gathered over the past seven years.

According to Under-Secretary of State Walter Stoessel, the evidence shows that the Soviet Union has been engaged with its allies in the use of weapons that are forbidden by the Biological and Toxin Weapons Convention of 1972. "The USSR is flagrantly and repeatedly violating international agreements, and this is now a threat to the whole international community, since toxin weapons are a cheap, convenient way of subduing and exterminating opposition which could be used against other people", Mr Stoessel said.

The State Department's report is intended to stem sustained criticism that it has failed to substantiate its allegations of the use of chemical and toxin weapons — and in particular of tricothecenes — with adequate medical and scientific data.

Various other hypotheses have been put forward to explain the presence of tricothecenes in samples which have been brought back from South-East Asia and

Incriminating data

Washington

The State Department report details how the evidence for the occurrence of mycotoxins has been obtained. It says that the US Army's Chemical Systems Laboratory was unable to detect them in the few samples returned from South-East Asia, so that Dr Chester J. Mirocha from the University of Minnesota was asked to apply his gel-separation and mass spectrometric techniques to the problem.

Three closely related mycotoxins are said to have been identified: T-2, nivalenol and deoxynivalenol. A sample of material thought to be contaminated and obtained from Kampuchea contained 109 p.p.m. of nivalenol, 59.1 p.p.m. of deoxynivalenol and 3.15 p.p.m. of T-2. Control samples of vegetation, submitted for analysis gave negative results.

Samples of water from Laos and Kampuchea contained 150 p.p.m. of and 25 p.p.m. diacetoxyscirpenol, another closely related toxin. A sample of yellow powder collected after a supposed attack by chemical weapons showed no evidence of toxins but did yield a yellow pigment, similar to one found by Dr Mirocha in a culture of Fusarium roseum. The report says that this may mean that the agents used in South-East Asia are crude extracts of Fusarium cultures.

A crucial element in the State Department's case is that the concentrations found in the samples from South-East Asia are greater than those associated with natural contamination. Typically, the latter yield only a few parts per million of mycotoxin, although one measurement of 41 p.p.m. of mycotoxin in contaminated grain in the United States is on record.

The report includes names of Soviet scientists and of four laboratories thought to be involved in research with mycotoxins. The laboratories are the Institute of Experimental Veterinary Science (Moscow), the Institute of Microbiology and Virology (Kiev), the Institute of Nutrition (Moscow) and the Institute of Epidemiology and Microbiology (Moscow). There is no suggestion that the research there is military in character.

analysed in US laboratories. Some have claimed, for example, that they could have come from various forms of rat poison, others that they might be the residue of naturally occurring fungus.

State Department officials, however, said at a press conference on Monday that they had looked closely at the various alternative explanations and had not been able to substantiate any.

Mr Richard Burt, the department's

director of political and military affairs, pointed to extracts from an East German military manual printed in the report which gives details of how toxin weapons might be used, pointing out that the suggested circumstances were similar to those reported in South-East Asia.

"We think Laos, Cambodia (Kampuchea) and Afghanistan are 'proving grounds' for testing the chemical and biological weapons capability of the Soviet Army", Mr Burt said. "In all three countries there is strong local resistance which stands in the way of Soviet objectives, and where the conventional use of troops would be very costly."

Department officials refused, on national security grounds, to say how all the various samples and reports had been obtained. In analysing the samples, Mr Burt said, the department had received technical cooperation from the British government as well as the Japanese.

Disputing press reports that tricothecenes were not powerful enough to be a useful tactical weapon, Dr Sharon Watson of the Army Surgeon General's Office said that experiments carried out at the army's testing centre in Fort Detrick, Maryland, had shown that haemorrhaging, caused by a severe impairment of blood-clotting, could occur at very low exposure levels. Experiments had shown that for a 70 kilogramme man the LD50 dose could be as low as 35 milligrammes.

Furthermore, Dr Watson said, the army had reason to believe that a crude extract from *Fusarium* was being used, which could be more toxic than the purified form. Referring to the broader implications of the charges being made against the Soviet Union, Mr Burt said that the evidence for the use of toxins in South-East Asia illustrated that one of the major flaws in the 1972 convention banning the use of toxins in war was that it contained no provision for verifying compliance.

David Dickson

Information technology

Cables coming

If Britain does not prepare to accept cable information systems now, then it may as well not bother at all. That is the message contained in a report to the Cabinet Office which was prepared by the government's Information Technology Advisory Panel and published earlier this week. Such is the urgency perceived by the panel of the need to provide cable television for British viewers that the report urges the government to make its intentions clear even before fully resolving some thorny problems such as regulation and licensing of the programmes that can be transmitted.

The government seems set to take on board the gist of the report's recommendations which include announcements of broad policy by mid-1982, regulatory arrangements by early 1983 and the

formulation of technical standards for the cable network by the end of this year. Civil servants in several government departments are now trying to work out the details. Thus the more leisurely approach to cable television which was envisaged as recently as early last year, when the Home Secretary approved 13 pilot schemes, looks like being abandoned. Decisions must now be taken, according to the report, before the results of those schemes can be known, in order to prevent overseas companies from hastening the decline of the British cable television industry.

The chief interest in cable systems is said to lie in their potential for linking new information technologies. But the panel believes that large numbers of users will only be attracted to the system quickly if it starts by offering a wide choice of television programmes. Mr Charles Read, chairman of the panel, hopes that programme providers can be licensed within existing legislation, which gives the Home Secretary wide powers of discretion.

Public fears about the quality of broadcast programmes, which traditionally have been tightly controlled in Britain, may not be so easily allayed. This problem will soon be tackled by Lord Hunt, formerly secretary to the Cabinet, who is to hold an urgent inquiry into the likely effects of cable television on the public broadcasting system.

Precise specifications for the cable system have yet to be established. But the panel's report envisages series of local networks, initially in large cities, that would offer broad bandwidth communications capable of carrying 15-24 channels. British packet-switching technology that would allow cable to individual homes to be of lower bandwidth than that on trunk lines is favoured.

The cost of cabling half the British population, according to the report, would be about £2,500 million, all of which would have to be found by the private sector, which apparently is eager to put up the money.

Costs, says the report, might be minimized if British Telecom ducts were used for laying the new cable. Local networks could, for example, be interconnected via its own telephone network. And as private broad bandwidth cables may well be installed before its own network is upgraded, the company would be wise to consider putting some of its own services, such as Prestel, onto the new cables.

While welcoming British Telecom's interest in the new cables systems, the panel's report is nevertheless cautious about the extent of the company's involvement. It is particularly keen, for example, that British Telecom should not dictate the standards for the new networks, presumably fearing that the company would be too restrictive and cause unnecessary delay.

Judy Redfearn

Neuroscience moves

New York

The Neurosciences Research Program, the seemingly clubby survival from the time when it seemed necessary, twenty years ago, to persuade people that neurobiology was interesting, is in the throes of moving from Boston to New York. At the same time, it has been given a new image and an income that it can call its own.

The organization was begun in 1962 by Professor Francis O. Schmitt, largely to proselytize on behalf of the neurosciences. Since then it has been housed in a replica of a French chateau 15 miles from Boston, and has been best known for its periodic small workshops (up to six a year) on various aspects of neurobiology. Now, however, the organization has negotiated a lease with Rockefeller University that will allow it to house not merely its administrative staff but a new institute, called the Neurosciences Institute, intended to provide up to half a dozen relatively senior people in the field (and perhaps as many junior colleagues) with an opportunity for conceptual (as distinct from experimental) work for short periods of time.

Both parties to the lease now signed are anxious to emphasize that the Neurosciences Research Foundation, by becoming a tenant of the university, will not become a part of it. The foundation will in future finance both the Neurosciences Institute and the Neurosciences Research Program from an income that appears to exceed \$500,000 a year.

In the move from Boston to New York, some care seems to have been taken to broaden representation in the management of the enterprise, with the result that it is often hard to tell who will do what. The director of the Neurosciences Research Program from the president of the foundation) is Dr Vernon B. Mountcastle, President of Johns Hopkins University. Dr W. Maxwell Cowan of the Salk Institute is the chairman of the Scientific Advisory Committee of the programme, but there is also a "scientific" chairman, Dr Gerald M. Edelman of Rockefeller University (who is also the director of the Neurosciences Institute).

Representation of the Salk Institute through Dr Cowan, is said to mark the plan that both the Neurosciences Research Program and the institute will be peripatetic, migrating en masse to the west coast for the summer months. Edelman hopes that the first of these summer programmes will take place this year, although formal arrangements with the Salk Institute have not yet been completed.

Polish crisis

Patriotic science

The role of science in overcoming Poland's economic difficulties will be a major point on the agenda of the next plenum of the Central Committee of the Polish United Workers' Party, Dr Hieronim Kubiak announced last week. Dr Kubiak, reputedly one of the most liberal members of the politburo, was addressing a national conference on "the role and tasks of science in getting the country out of the economic crisis".

Dr Kubiak stressed that "although science has no country, scientists do have countries", and appealed to the patriotic sentiments of all Polish scientists, asking them to rally to the new economic strategies.

From the Academy of Sciences, Dr Zdzislaw Kaczmarek, the scientific secretary, who holds quasi-ministerial rank and is accountable directly to the prime minister, announced a new government act, which will define the legal obligations implicit in scientific consultancy. Not every researcher, he said, has the qualities needed by a consultant, such as the ability to translate the language of science into the language of politics, and, most important of all, the courage to oppose "irrelevant" pressures.

The timing of the meeting was not without irony. On the same day, the Central Committee Commission for Health and Environment discussed a report on the disastrous level of environmental pollution - a subject which, until September 1980 could only be discussed in clandestine unofficial pamphlets. Even during the 16 months of Solidarity, the most revealing data, including the fluorine pollution of the Krakow area, could only be discussed in "internal" bulletins of the Polish Ecological Club. Yet now the commission cited "lack of adequate education" and information as a major cause of the current pollution levels. A few days before these meetings, another report had been under discussion in the Polish media discussion of the factors leading up to the imposition of martial law, produced by the "Experience and Future" group (Doswiadczenie i Przyslosc, or DiP), an unofficial working party of intellectuals set up in 1978 to discuss the state of the country. In spite of official disapproval, DiP produced several reports on the economic crisis and proposed strategies for overcoming it. The latest such report, drawn up on 20 December, was ignored by the authorities until early March, when it was officially condemned as a Western forgery - although the same commentators then proceeded to vilify Stefan Bratkowski, head of DiP and chairman of the now-suspended Union of Polish Journalists. At least 30 scientists actively participated in DiP and have thus,

effectively, had their proposals for the economy already rejected. How far they are liable to respond to Kubiak's patriotic appeals is unknown.

One notable figure, however, was absent from the meeting with Kubiak - Dr Aleksander Gieysztor, president of the Polish Academy of Sciences. He had been allowed to travel to Paris, to be honoured by a learned society for his contributions to historical research. The Paris ceremony was kept deliberately low-key, since Dr Gieysztor had made it clear that he was present solely in his private capacity, and not as a representative of the Polish Vera Rich scientific establishments.

Universities in commerce

Stanford's way

Graduate students at Stanford University, denied entry to this week's conference on academic-industrial relations organized by the university's president, Dr Donald T. Kennedy, are holding a rival conference of their own in public. The Stanford graduate students' association has organized one meeting for 26 April and another for 10 May. The students' objectives are to help define their own reactions to the involvement of outside commercial interests in academic research and towards academic supervisors who acquire equity and managerial positions with companies whose interests are related to their research.

Kennedy has agreed to be the keynote speaker at the first of the students' meetings. Professor David Noble of the Massachusetts Institute of Science and Technology and author of American by Design, a study of the links between science and technology and the growth of corporations, will also be speaking.

For Kennedy's own meeting this week the presidents of Harvard University, the California Institute of Technology, MIT, the University of California and Stanford, together with teams of academic colleagues and industrial partners, are turning up at Pajaro Dunes. A possible code of practice to regulate the relationships between universities and commercial interests has been circulated, but even if this or some amended code is agreed on, the result will still have to be discussed within the faculties of the five universities.

One of Kennedy's messages for the students is likely to be that industrial corporations can contribute both financially and technically to the education of graduate students. He will be able to point to the arrangement now concluded between Stanford's new Center for Information Systems and seventeen major electronics companies, which will contribute \$250,000 a year for each of the next three years towards the general running

Meanwhile, Stanford is taking steps to

Joint Research Centre

Jean-Albert Dinkespiler, an old hand at European research efforts, is to take over from Professor Stelio Villani as Director-General of the European Community's four "Joint Research Centres". The four centres at Ispra (Italy), Geel (Belgium), Karlsruhe (West Germany) and Petten (Netherlands) employ about 2,000 European scientists, and boast a budget for the period 1980-83 of 510 million European Currency Units (£287 million).



Dinkespiler, a 55-year-old Frenchman, has already seen active service at the French national centre for space studies, at the European Space Agency, as Deputy director-general of the Joint Research Centres and head of Ispra and most recently as director-general responsible for science, technology and energy at the Council of Ministers.

Jasper Becker

regularize the distribution and use of what is called "tangible research property" unpatentable products of research which may, nevertheless, have commercial value. New guidelines for the distribution and use by others than the original scientist have been drawn up partly to draw the attention of investigators to the possible commercial importance of such things as cell lines, computer data bases and software. circuit diagrams and engineering drawings.

Dr Gerald J. Lieberman, vice-provost at Stanford, says that investigators who create tangible research property should ordinarily be the ones who decide how it should be distributed or used. Such property should not be sold for profit, but steps should be taken to see that the assistance provided by academics at Stanford is afterwards recognizable (and, if necessary, acknowledged in writing). The objective seems to be to encourage researchers to assist their colleagues elsewhere while not hazarding the university's right to control the exploitation of those developments which turn out to have commercial value.

Charlotte Beyers

CORRESPONDENCE

Creation deduced

SIR — Having recently received, for the first time, a communication from the Biblical Creation Society which contained quotations both from my letter to you of last July (*Nature* 30 July 1981, p.403) and from Dr O'Grady's comments on it (*Nature* 10 December 1981, p 510), I am reminded that a short rejoinder to the latter is needed.

It was kind of O'Grady to think that I needed information about the writings of Messrs Hume and Popper on deductive and inductive thinking. However, what I was trying to do - perhaps not clearly enough was to put into perspective the original dichotomy between the two modes of explaining things, a division which must surely go back, as I said, to the earliest days of man as a toolmaker. In those times deduction must have more or less automatically meant a preconceived belief in, and an assertion of the truth of, dogmas and myths. Only, I suggest, with the emergence of properly understood scientific reasoning within the past few centuries, involving the testing of ideas, has deduction acquired its constructive aspect and become an alternative to induction in the formulation of hypotheses which make testable predictions.

My main point was that the original "prehistoric" form of deductive thinking is still with us and flourishing - perpetuating many myths both ancient and modern. I believe "creation science" is a classic example of this as it seems only concerned to propagate, in the face of a mountain of contrary evidence, a 3,000 year old myth about the origin of the Earth and of man. If this is not so, and if creationism is scientific. deductive thinking, someone will have set out the creationist hypothesis in much more detail than appears in Genesis and will have tested it sufficiently often and successfully to think it worth continuing. Has this happened? Perhaps the three Glasgow University biochemists (see Nature 26 November 1981, p.302) have done this as well as simply stating, rather negatively, that the evidence does not disprove the existence of a Creator?

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Kindly explained

Sir - Marks1 as well as his creationist critics²⁻⁵ seem to be arguing at cross purposes. Unlike Marks, one ought not to sneer at the views of fundamentalists, just as a clinician must not disregard the delusions of a psychotic. Both types of belief mean much to those who hold them A creationist cannot be convinced by facts or scientific arguments of any possible errors of an a priori infallible creed, any more than a psychotic can be shaken about the absolute truth of his delusions by rational counter-arguments. When contrasting science and creationism we must remember that we are dealing with different kinds of explanations concerning the world. Let me paraphrase in an evolutionary context a passage from the late C.A. Mace written in a behaviour-scientific context in an editorial foreword to a book by Vernon⁶.

"The difference between evolutionists on the one hand and creationists on the other arises perhaps from a failure of the latter to do justice to the fact that there are many different kinds of explanations. There is the kind of explanation that fossils exist, which simply says that fossils are remains of past organisms, irrespective of whether these organisms were divinely created or arose by self-organization. This is a neural kind of explanation. There is the sort of explanation which appears to satisfy evolutionists and some other life scientists, which takes the form of saying that fossils are relevant to our formulations of particular theories of evolution-specific mechanisms7. There is another kind of explanation which satisfies materialisticallyminded mechanists, who (like myself⁶) suggest that fossils are the remains of organisms that arose by self-organization. Perhaps also some notice should be taken of the kind of explanation which satisfied children and devout old ladies who would say that fossils exist because God arranged things so as to remind us of the many beautifully adapted organisms that he has created in the past.' There is room for all of these views among the right people in the right places and in mutually exclusive ways. We must remember that different kinds of explanations rely on different premises and on different explanatory procedures. Thus, hypotheticodeductive scientific theories are not intended to proclaim absolute truths, but are, or should be, falsifiable. By contrast, the doctrines of creationists are proclaimed as absolute truths of a metaphysical kind, and do not function like scientific hypotheses. If scientific facts do not fit creationist doctrine then, with sufficient ingenuity, an indefinite number of reinterpretations of the doctrinal metaphysical statements remain possible. In the Popperian sense there exists an unlimited range of built-in metaphysical defences of fundamentalist doctrines. It is therefore not uncommon for scientists to accept two schemes which may be related to the same facts. On the one hand they may accept hypothetico-deductive theories of mechanisms. On the other hand they may embrace a metaphysical belief system which explains some of the facts known to them from science in ways totally different from the ways in which they occur within scientific theories. I know many such people and some highly intelligent ones among them, although I see no personal need to supplement science with a fundamentalist metaphysics. (Some kind of metaphysics is probably needed

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1. Marks, J Nature 295, 276 (1982).

in science⁹.)

- 2 Cousins, F W Nature 295, 452 (1982)
- 3. McBride, N.K & Kreitzer, L. Nature 296, 8 (1982)
- 4 Hollin, A J. Nature 295, 548 (1982)
- 5. Hamblin, T H Nature 296, 108 (1982)
- Vernon, M.D. The Psychology of Perception (Penguin, London, 1962).
- 7. Wassermann, G D Phil. Sci. 48, 416-437 (1981).
- 8. Wassermann, G.D. J. theor. Biol 96 (in the press)
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Aptly put

Sir — The comparison of spontaneous development of life to the creation of a Boeing 747 by a "tornado sweeping through a junkyard", cited by Jukes (see Nature 18 February, p.548) as being inept, is, on the contrary, very apt. Probably purposely, but possibly inadvertently, Jukes fails to identify the Wright brothers, Dumont and Blériot as creators. Every additional step in the development of the 747 is a result of creative minds. Without such creative genius, and with or without any number of tornadoes, a junkyard is still junk, albeit with useful materials for creative minds; and without a Creator, atoms and molecules, which He created, are still only atoms and molecules. (Go back farther to matter and energy, if you wish.) In referring to the creators of the modern aircraft (those he mentions and their successors), Jukes has most elegantly refuted his own argument.

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The real dangers

SIR — With regard to the correspondence from Chris Q. Doe (Nature 4 March, p.8), I think it is Mr Doe who is demonstrating "extreme ignorance". The statement that "any amount of radiation will produce a proportional increase in mutation rate" is an unproven supposition, and a highly doubtful one in view of recent results on the self-repairing abilities of DNA (of which Mr Doe should be aware if he is really a biologist)

As for the relative dangers of hydroelectric versus nuclear power plants, it is a fact that several thousands of people have been killed by collapsing dams during the past twenty years alone, while the number of fatal casualties caused by nuclear power plants can be counted on the fingers, not a single one occurring to people outside the plants (except possibly in accidents due to construction work traffic). The increased mutation rate caused by nuclear plants is, again, highly hypothetical; even if it exists, it cannot amount to more than a fraction of a per cent of the rate caused by natural radiation, itself a small fraction of the rate of mutations occurring spontaneously or caused by chemical agents. Who says that mutations are necessarily harmful, anyway? Darwin would have thought well of them had he known about them

What science has to do with the soundness of decisions to build nuclear power plants is beyond me; as for the economics of it I leave that to the electric utilities, who presumably aren't building them just for the fun of it. Living in a country which is seeing its forests and lakes rapidly being destroyed by acid rain (already the fish are gone from 10,000 lakes in Sweden, many are practically devoid of life), I have no doubt at all, however, that nuclear power is the environmentally soundest way of producing the energy we cannot do without, and by far the one doing the least harm to nature, both momentary and permanent, and with all aspects considered.

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SCIENCE IN FRANCE

The grand experiment

SCIENCE in France is going for boom — or bust. The objective is to be the (socialist) Japan of Europe, and new technologies, founded on good science, will lead the way. What a contrast with the rest of the developed world. It is a great experiment, which deserves to succeed. Of course, along the way there will be many pitfalls, and in the next few pages an attempt is made to reach an objective assessment of the situation.

In fact, the experiment is not a new one. French science and technology returned to the centre of the world stage in the 1960s and 1970s thanks largely to the vision of General de Gaulle. According to figures collected by one of the present science minister's cabinet advisers, M. Pierre Papon, French science spending didn't just increase during de Gaulle's rule — it quintupled. In 1958, the French government spent FF1,540 million on research and development; and in 1967 (when de Gaulle resigned) the figure was FF8,836 million. Part of the rise can be accounted for by inflation, but even as a fraction of the government budget, the increase was staggering; from 2.5 per cent in 1958 to 6.2 per cent in 1967.

De Gaulle sought national independence, which among other things implied economic and military independence of the United States. There should be French nuclear weapons; French rockets; French electronics; French energy. And of course, French science both to support this technological development and, not least, to fly as a flag of French pride.

The experiment worked, in most essential areas (electronics still has a long way to go); and in the process science in France was transformed. There were 9,000 researchers in the state sector in 1958, and 21,000 in 1965. (The 1968 university revolution, which came the year after de Gaulle's departure, added even more: the figure was 31,000 in 1969.) This was the age of gold.

As if to confirm de Gaulle's investment, though no doubt entirely unconnected, the first of six Nobel Prizes won by Frenchmen since the war were announced in 1965: those of André Lwoff, François Jacob and Jacques Monod. Monod, however, gave a sour interview to the weekly newspaper Le Nouvel Observateur after his award, claiming that M. Georges Pompidou, de Gaulle's prime minister, was not doing enough for science. ("Pompidou never forgave him for that", says Jacob.) Perhaps it was true that more was being done for physics than biology, though one of those concerned with distributing the money in those days - M. Charles Thiebault, recently president of the Centre National de la Research Scientifique - says molecular and cell biology had a high priority in de Gaulle's programme. Thiebault's figures on French research productivity in the 1970s (see following page) show that the most dramatic increases in that decade came in molecular and cellular pharmacology, a fact which can be traced back, he believes, to increased support in the 1960s.

But how long can such experiments last? By the 1970s, the gold had tarnished. De Gaulle had pushed the national French research and development effort, including industry, from under one per cent of gross national product in 1958 to 2.2 per cent in 1967. Monod seems to have been right about Georges Pompidou, for when he became President the science budget began to fall. By

1980, research and development spending accounted for only 1.8 per cent of the country's gross national product. French science during the 1970s was based largely on the structure and staff given to it by de Gaulle.

Today, the unlikely inheritor of de Gaulle's scientific mantle is the left-wing socialist M. Jean-Pierre Chevenement, not as president of France, but as minister of state for research and technology with his own powerful ministry (something de Gaulle, in fact, avoided setting up). Chevenement, strongly backed by President François Mitterrand, is in effect relaunching Gaullism for science — albeit with a human face. The difference is that Chevenement's programmes are being designed for the people; de Gaulle's were for "France". If the difference seems subtle, that's because it is subtle. There is only a touch of democracy in Chevenement's thinking. His is a planned economy. But it is one, at least, in which the impact of technological change on the fabric of life in France will be taken into account in the development of policies.

Also, and inevitably in 1982, Chevenement's choice of grands programmes differs from de Gaulle's. The new minister does not control defence research (which was 65 per cent of de Gaulle's first research and development budget). So physical science will not be so prominent. In fact, the most advanced and active of Chevenement's plans concern biotechnology, with its spin-off of support for molecular biology, microbiology and enzymology.

The 64 million franc question, however, is whether the plan will work at all. The whole economic exercise now under way in France is a gamble on investment: the exact opposite, say, of British policy. The projected budget deficit of the French government for 1983 is now FF 200,000 million (£20,000 million). Moreover there are structural and psychological obstacles to the further development of science — and in particular, technology — in France. These centre around the strong, historic distinction that has been drawn in French higher education between training (represented by the grandes écoles) and culture (represented by the universities). De Gaulle probably went as far as he could without changing this system substantially. Chevènement, needing to go beyond de Gaulle's achievements, may not have that luxury.

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This supplement has been compiled by Robert Walgate, Nature's Chief European Correspondent, and written by him unless otherwise indicated.

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Has France got the money.

IT seems that France is now committed to an immense and breathtaking economic gamble, like a hardened punter throwing all his chips on one spin of the wheel. In direct contrast to the straitlaced grocershop monetarism now the fashion in Britain and the United States, the French solution to the recession, to unemployment and the challenge of Japan is to spend, spend, spend.

Consider the ministry of industry. Capital aid to industry and business excluding the defence industry and support for research and development - has grown 80 per cent this year to FF8,000 million (£800 million). Against such massive increases in direct support to industry, the 29 per cent increase in the government research budget for 1982 looks almost niggardly. Moreover, the increase in spending money - as opposed to commitments to order, but not actually buy, equipment and materials - has risen only 23 per cent, or 9 per cent allowing for current inflation; and since the US dollar has risen 50 per cent against the franc in the past two years, and many materials and much equipment must be bought at dollar prices, some laboratories claim that they are at best maintaining their real level of expenditure. Furthermore, some 25 per cent of this year's new money may be withheld if the economy does not perform as hoped, so there are growing doubts among researchers as to the true value of the "boom".

Nevertheless, Jean-Pierre Chevènement, minister for science and technology, is giving major boosts to certain areas, which must survive the worst of disasters. ANVAR, the Agence Nationale de Valorisation de la Recherche, which seeks to convert research in university and government laboratories into productive industrial innovations, will increase its budget by 76 per cent this year; alternative energy research grows 50 per cent; and aid for the dissemination of scientific information, up 80 per cent.

Meanwhile the budget and foreign trade deficits balloon massively. The total government budget will increase by 28 per cent this year - leaving an estimated deficit of FF95,000 million (£9,500 million). The foreign trade balance (dominated by oil imports) is expected to be in deficit to about FF60,000 million (£6,000 million). For rough comparison, Britain's current account deficit is about 10 times less severe, when oil revenues are subtracted. (Including oil revenues, Britain is in the black to about the same degree that France is in the red.) around: the Mitterrand gamble is a long one.

and the science?

FRENCH science is on the move - upwards - according to figures prepared by the expresident of the Centre National de la Recherche Scientifique, Professor Charles Thiebault, with help from CNRS and the Pascal bibliographic data bank (see table).

Thiebault calculated the percentage of research papers of French (and other non-US) origin in the 286 most-cited journals of Science Citation Index, and compared two years as near as possible to 1970 and 1980. By this measure, all French science except physics increased in literature penetration during the 'seventies. The best single penetration at the end of the decade was achieved by French astronomy: in 1979, 10.8 per cent of the surveyed astronomy papers were French. The best improvement was in pharmacology, from 0.5 to 5.5 per cent. Most of the papers were in English.

By comparison, the United Kingdom suffered a dramatic decline.

As with all such figures, however, the data must be interpreted with caution. The number of French scientists leapt up during the expansion of the universities in 1968-71, so to some extent increased penetration is a measure of increased quantity rather than quality. For example, French mathematics is certainly far better than seems to be indicated by its penetration of the literature. Since 1945, the Fields medal for mathematics - the

"Nobel Prize" of the subject - has gone five times to France, compared with only eight to the United States (which has five times the population). By contrast, in the scientific Nobel Prizes, France has been weak: six since 1945 (two in physics, four in physiology and medicine) compared with

13 in Germany and 35 in Britain.

Praction of papers (%) in major scientific journess								
Disciplines	France	Germany	UX	Japan	Canada			
Mathematics								
1970	3 4	90	10.0	1.0	4.5			
1988	7.2	11 5	6.9	3.3	3 4			
Physics								
1973	8 5	6.4	10.2	4.0	4.5			
1979	8.2	8.4	67	5.3	4.3			
Engmeering								
1972	4.5	47	15.5	46	56			
1979	59	5 5	8.8	8.1	4 2			
Chemistry								
1970	2.9	3.7	16 2	5 9	4.2			
1988	73	5.8	10.5	8 8	4.5			
Geophysics/Space								
1972	3 5	3.1	10.4	19	7.1			
1979	5 0	3.7	9.3	2 4	6.6			
Biology								
1970-73	2.3	46	16.0	2 8	39			
1979-80	4.4	50	12 0	4.7	4.6			

Thiebault himself interprets the figures as a good broad measure of the productivity of the community of French scientists, indicating that France is now producing science "at the normal rate". But he is worried about what will happen in the late 1980s. Scientists are most productive in their 30s and 40s, Thiebault believes, and the drastic fall in French recruitment of young scientists in the 1970s may ultimately show through in output.

Read right on from here

In the following articles, there is an Anglo-Saxon look at France in something approaching a logical sequence. But it may not be logical enough for a French man or woman. Logic is a great French strength, but also a weakness. The greatest respect goes to the most abstract disciplines: purest of pure mathematics, the most theoretical anthropology. The schools create this love of logic, and the grandes écoles foster it.

Logic is also linear, and the French certainly love the direct route. Crossfertilization and mobility seem to be foreign ideas. The author of a recent report recommending the establishment of an agency for technology assessment wrote "in France, there are traditional difficulties in thinking in terms of complex systems and networks . . . ".

Careers also are seen linearly. A vogue word these days is filière, which is usually applied to industry: a filière is a whole chain of connections which takes a primary material to its marketable product. There are filière electronique, filière énergetique, and filière what-youwill. Industry is a clutch of distinct filières. It is the same with careers. One follows one's chosen career, with a series of qualifications marking progress and serving to attach one firmly to this line rather than another.

In the following pages, some of these threads are laid out: the line of the grandes écoles, which leads to industry and the ministries, and skims off many of the most able pupils; the line of the universities, still wavering after the "revolutions" of 1968, where most French research is done; and the line of the grands organismes, the big state institutions which fund and control the best of that research.

The new politics of research are outlined on page 299 — the minister for science, Jean-Pierre Chevenement, wishes to push French research and development spending up by 25 per cent in real terms by 1985, to 2.5 per cent of the gross national product. Chevènement may be spending more money and employing more people, but he is also attempting to link a few of the stubbornly separated filières: to link industry with the grands organismes, research with the grandes écoles, thereby seeking greater productivity from French science.

At the same time, Chevènement will be creating some filières of his own: a number of full-scale national research and development programmes, after the style of de Gaulle before him. We sketch one: biotechnology (page 295). Finally, we review two of the most successful existing French technological programmes: space and nuclear energy.

Great schools, great contradictions

THE most extraordinary thing about the French education system is that it is dominated by science, and yet it is inefficient at producing scientists. At the age of 14, children are selected to study for this or that branch of the baccalauréat, or "bac". The most able are groomed for bac C: mathematics and physical science. To do anything else is, by comparison, to opt out of the academic race. Bac C pupils are virtually the only entrants to the grandes écoles, the higher education production lines of the French élite. When bac C pupils go to university, as nearly half of them do, they do better than any other class.

Yet of this leading group, only a minority choose to study science. Have they had enough of it? Roughly one in eight go on to short-term technology courses at "instituts universitaires de technologie". Another one in eight (about 3,000 a year) study science at university. Two-thirds of those who go to university study something other than science.

The contradiction goes deeper. The really ambitious bac C pupil goes on to two-year preparatory classes, cramming schools aimed at the special entrance exam to the grandes écoles, the concours. The preparatory courses are called taupe (and hypotaupe) — that is, "mole" and "hypomole" — for good reason. The work is so intense that the student never lifts his or her head to the light of day. Once a student is successful in the concours, the grandes écoles themselves are a relaxation: a qualification and a good job are almost guaranteed.

What then do these moles learn when they emerge into the grandes écoles? Not "science", properly speaking. The teachers, on the whole, do not do research. There are great variations in coverage, and in quality, but what is called "engineering" dominates. Of 300 or so grandes écoles, 160 are engineering schools. Another 60 are business schools, and another 80 cover miscellaneous arts and occupations. Two or three schools do have substantial research departments, including the prestigious Ecole Normale Supérieure, and so teach science effectively - but only to a few hundred, in total, of the bac C élite. Moreover the science is almost exclusively physical; biology has until recently been largely neglected.

Even the engineering taught by the grandes écoles is said to be too theoretical, too dry and too dated to be good engineering (though there are important exceptions, of course). So what do the grandes écoles teach their students? They teach rigour in argument, and — a practical matter — how to take decisions.

The subjects studied might as well be classics for all the significance they have. The jobs pages of *Le Monde*, the principal French newspaper, are plastered with adverts for *ingenieurs grandes écoles* not

because these are engineers, but because these will be the most intelligent managers. The ministeries of the French state also recruit most of their top staff from the grandes écoles.

The remarkable result is that while French education stresses science from the age of 14, the product is a group of managers and civil servants who could hardly be further from research. The university system, where research is done, is seen very much as second class, and the social distinction is maintained throughout life. The club of the grandes écoles is exclusive, and the damaging consequence is that research penetrates industry far less than it should.

The universities themselves have their own problems. They were not ready for the student population bulge of the sixties, and together with certain social and political factors the result was the transformation of 1968, whose impact—has still not been properly assimilated.

The rise in student numbers was dramatic. In 1960, there were 223,000 students studying in the French universities — which were then arranged as almost totally independent, unconnected faculties specializing in this or that discipline. By





(Plantu, Le Monde)

1968, there were more than 575,000 students cramming into the same lecture halls. "Often there were hundreds at a lecture", wrote one observer, "many of them sitting on the floor right up to the blackboard. Students had to arrive early to get a place". The chalky lectures seemed out of touch with the concerns of youth: the Vietnam war, alternatives to "scientism" and economic growth. The students demanded change, and got it.

The present French universities, apart from ancient establishments like the University of Paris, are largely the creations of 1968. Faculties were grouped into multidisciplinary campuses. Students were given rights in university administration, new courses started, new



First lady at the École Polytechnique: Anne Chopinet graduates in 1972, one of a group of seven who were the first women ever to study there. Now she deals with mineral resources and uranium exploration at the ministry of industry.

universities founded, and new lecturers recruited. Recruitment had been going on earlier, however. In 1960, there were 10,000 university teaching staff from professors through maîtres conference, maîtres assistants, to assistants. In 1967-68, the number had reached 26,000. Some 2,000 assistants were recruited that year. But in 1968-69, the number of appointments was 4,500; in 1969-70, 2,500; and in 1970-71 (slowing down substantially) 800. By 1973-4 the number of teaching staff was nearly 41,000 (where it has roughly remained for ten years). Thus in 1980 there were 41,000 lecturers and 861,000 students, a university system almost exactly four times that of twenty years before, containing a mass of assistants created in one brief five-year period. Throughout this higher education boom, the grandes écoles remained aloof, taking almost constant numbers.

The suddenness of this growth, and the continuing strong position of the grandes écoles, has meant that the universities have found themselves almost entirely detached from the centres of power in France, from industry and from government (which nevertheless keeps them on a tight rein, like an errant child). There has thus grown up a feeling — in part a legacy of 1968 — that the university and its research should not be sullied by contacts with industry. This has become a principle, one which also gives the new university system a feeling of dignity in French life.

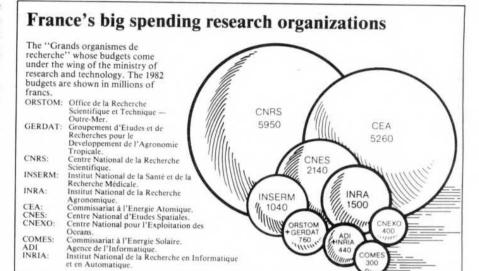
The distinction is even reinforced by law: university staff are civil servants who, in principle, should not seek consultancies in industry; and the universities should not enter into any kind of profitable liaison with a company. Needless to say, the more enterprising universities and staff have set up such links by devious means; but the law sets up obstacles. (One of the most important exercises undertaken by the new

minister of science, Jean-Pierre Chevenement, therefore, will be to remove these legal trip-wires, and the forthcoming "law of research" (see page 289) should do so. But the established suspicion of industry will remain.)

There is also another weakness in the French university system: its relation to research itself. The control and management of French science rests essentially with the grands organismes, roughly equivalent to other countries' research councils, but more powerful and paying more salaries. The grands organismes draw their researchers from the university system, give them better research facilities, remove their heavy load of teaching and administration but - for the moment - give them worse conditions of employment: lower pay and fewer rights, even though they may be working in the same campus, even the same laboratory as university "teacher-researchers"

Most scientists, however, are keen to make the deal — as is evidenced by the nearly eight-to-one ratio of applicants to posts at the junior grands organismes level of attaché (see page 287 for details of the more prominent grands organismes). The grands organismes, plus one or two separate research institutions like the Institut Pasteur (biology) and the Institut des Hautes Etudes Scientifiques (mathematics and theoretical physics) are the heart of science in France.

Now comes the crunch: since grands organismes researchers tend not to teach,



and since they do the best research, higher education is denuded of its leading scientists. So how are scientists trained?

This may become one of the most important issues in French science policy, for with the promised civil servant status for grands organismes researchers, matching that for university teachers, there will be nothing to keep researchers in the lecture theatres but a dedication to teaching. Much is now being made of the catch-phrase "education for and through research", but the means are still being sought to bring it to reality. The grands organismes are now under pressure to play a greater role in education, and they must

take it up if they are to find the scientists they need in future years — but the universities are very wary of such moves, seeing them as an encroachment on the territory.

All in all, there is a lot to be done. The government does seem to be aware of the problem: the minister of education, M. Alain Savary, promises a new law for the universities by the autumn, and he is working in close collaboration with his pushy opposite number in the ministry of research, Jean-Pierre Chevenement. We shall see then if anything substantial and long-lasting is to be achieved with French science.

What Aigrain thinks of it all

THE man who must look most wryly at the present efforts to create a renaissance in French science and technology is Pierre Aigrain, now director of research and development at Thomson CSF, but minister of science under Giscard d'Estaing.

Wryly, because he had begun to push up the science budget in 1981 (not so much as Chevènement in 1982, but the latter's efforts are carried on the tide of an overall 27.6 per cent increase in government spending). Wryly, because the politics of opening science to industry were his own. In some areas Chevènement has even taken on the very advisers that Aigrain used. Chevènement appears to be getting the glory for a policy which, in large part, was established by Aigrain. So what does he think of his successor's plans?

Aigrain is "very glad" about the proposal for a law that would more or less guarantee the science budget for a few years (see opposite). But otherwise he feels there has been a change of wording more than a change of substance. Aigrain clearly thinks that the National Colloquium on science and technology, which drew together scientists and others influenced by their work in a great

jamboree of meetings late in 1981 and early this year, was a marvellous idea, for the real problem of development in France is a matter of psychology as much as one of regulations and budgets; the colloquium demolished some barriers.

However, he is worried about Chevènement's accelerations of scientific recruitment to the grands organismes



Pierre Aigrain — pleased with the "loi programme" but worried about industry

(like CNRS) from 3 per cent of staff per year (under his ministry) to 4.5 per cent. There are enough good people to fill the quota, says Aigrain, but not enough left over to satisfy the needs of science-based industry; and since the security and terms of employment in the grands organismes are excellent, industry is going to have a

hard time reaching the 8 per cent a year real growth in research and development demanded by the new minister.

Education needs an overhaul, and the government is aware of the problem, Aigrain believes, but an education system has a lot of inertia. Moreover, the source of the problem lies in the secondary schools, so change effected there will take ten years to have an impact on research.

The secondary schools overstress the mathematical and physical sciences, are too abstract, and demand too much of their pupils, he says, "so people who would have been quite good physicists and electrical engineers turn to biology to avoid the 'terrorist' attitude of our mathematicians". The result is too many biologists and far too many social scientists.

The grandes écoles, the majority of which teach engineering, also have a lot of drawbacks. They are rather small, and so produce too few engineers. On the other hand, the universities have completely neglected to train people for industry, and although good research is done, little of it is relevant to industrial problems. "What should be done is to improve the university system and its links to industry, and the selection of university students", Aigrain concludes.

Judy Redfearn & Robert Walgate

No union dominance at the CNRS

The minister answers some questions

THE new politics of research and development in France raises many questions, but some are particularly troublesome. How far will Jean-Pierre Chevènement, research and technology minister, go along with trade union demands for democracy in the laboratory? Why does he insist on the importance of French as a scientific language? And finally, what can he do about getting more scientists into industry?

The first question is particularly topical, for the scientific trade unions in the Centre National de la Recherche Scientifique (CNRS), the leading basic research institution in France, have buried their differences to demand union, and only union, representation on the Comité National — the all-important "parliament" of CNRS (see "Pressing problems for Payan", page 293). What does Chevènement think of that Robert Walgate asked him?

The minister says he is at pains to preserve the "effectiveness and openness" of the Comité National, which must play a greater role than before in determining the policies of the CNRS. But he added that although there would be "no question" of giving a monopoly of seats to the unions, the seats should be open to the unions (through election). It would also be a good thing, says Chevenement, if the unions began to concern themselves more with "the quality of research" in their politics, a remark that indicates that he would like the union to broaden their horizon. Nevertheless, says the minister, a union presence "raises the level of debate".

As for promoting the use of French in science, Chevenement says the objective is to help communicate science to the public, and to those who might make use of it in industry, "to maintain the strict link between the French scientific community, and the national community, which would be put under great strain if French ceased to be the language of scientists . . . French must be defended even in areas of research where the rule is to use English."

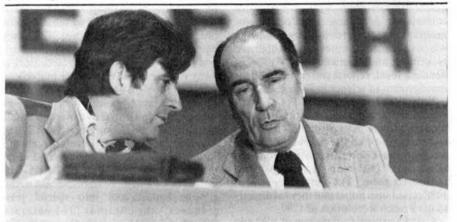
It follows that all French research must be published "at least" in French and, "if possible", in other languages, says the minister. In conferences held on French soil, French must be one of the working languages. A long-term programme must be undertaken to "renew French scientific and technical publications". A government supported publishing house will be set up for this purpose, says Chevènement and there will be "a vast effort" to translate foreign publications into French.

Many have claimed that the cost of such

a programme will make it impractical, but "it will not cost as much as they say" says the minister. The budget for the programme this year is FF 70 million (£7 million). But Chevenement adds that this budget is planned to grow "considerably" in the years to come.

Finally, research in industry will be supported by plans to train 1,500 engineers a year "through research" by 1985, compared to only 500 a year at present. (France produces some 11,000 qualified

engineers a year at the moment, but only a small fraction have training in research.) Particular efforts will be made to train the engineers and scientists needed for the *programmes mobilisateurs*—the special long-term programmes of the ministry, such as biotechnology and electronics. "This is in line with a major policy of training through research that we think will resolve the problems of high-level scientific recruitment, both for the public research organizations and for industry".



"Make France the third scientific and technological power of the world" François Mitterrand (right), President of France, has told minister of state for research and technology, Jean-Pierre Chevènement (left).

A new law for science

ONE law for the military, one for science: the management of the defence budget was foremost in the science minister's mind when he created a "programme law" for science which would define the budget and orientation of his ministry for five years. The ministry of defence employs a series of such laws, which avoid the frequent battles for funds and policies.

The result is the "loi de programmation et d'orientiation", now before the French economic and social committee for comment and possible amendment. The law fixes a government civil research and development budget increase of 17.8 per cent a year in real terms, averaged over the three years covered by the law. Fundamental research gets a 13 per cent per year increase. Jobs in science will be created at the rate of 4.5 per cent of existing positions a year (on average). And support will be given to industry to increase research budgets by 8 per cent a year.

No details are given in the law of how the money is to be spent, which has been taken by some to be a weakness; on the other hand, the ministry sees this lack of detail as a strength, giving it room for manoeuvre.

Moreover, one major financial battle has already been won: in the section on grands organismes the law speaks of financial control a posteriori. Up to now the ministry of finance has held very tight rein on the details of budgets with laboratory directors consequently unable to make even minor changes in spending once the budget for a year has been agreed. Under the new law, there should be more freedom.

This most important section of the law also redefines the role of the grands organismes. They would now have four tasks: to pursue research in every domain; to ensure discoveries are brought to application wherever possible; to spread knowledge; and to educate and train people "through and for" research. The law would also give the grands organismes a better legal framework in which to cooperate with industry. This would allow CNRS, for example, to join with and even hold shares in sister companies.

Researchers in the grands organismes, however suspicious they might be of industry should welcome the provisions in the law for a new contract of employment which would give them something approaching civil servant status.

Other important provisions in the law are an emphasis on regionalism and an identification of the main programmes of research to be developed by the ministry. These are: the rational use of energy and new sources; biotechnology; electronics; research in cooperation with and for the Third World; research on working conditions and the impact of new technologies; the promotion of French as a language of science; and technologies related to French industrial development not included in the above (with the exception of nuclear engineering).

Science — the next revolution?

THE Mitterrand government wants to make its mark on history. So for what will we remember the minister of state for research and technology, Jean-Pierre Chevènement?

Quite likely for what the newspapers dubbed the "States General" of science, an apparently formless meeting in January this year of some 3,000 French men and women representing almost every nook and corner of French science and technology. This National Colloquium on Science and Technology was merely the culmination of a large series of regional meetings — the Assizes Regionales — which took place the previous October and November, and generated 200,000 pages of detailed, local, reports and criticism on the management of science in France.

Why "States General"? Les états généraux were a feudal institution, last used by Louis XVI in 1788, in which reprentatives of all the parishes of France were called to Court to make their concerns known to the king. Louis himself misjudged them. The States General of 1788 raised such hopes that they led directly to the French Revolution of 1789.

So the National Colloquium of 1982 was a "States General" of science. That's not to suggest that next year there will be a revolution — but great hopes and fears have been raised among the scientific masses, and Chevènement now has to ride this flood or be submerged. Already certain trade unions, strong in French science, are threatening a struggle (see below). Whatever happens to Chevènement, he will be remembered for having created the National Colloquium. Pierre Aigrain, Chevènement's predecessor as

minister of science, clearly regrets that he did not think of the idea himself. . . .

So what are the main currents at work? It's not possible here to do justice to the 200,000 pages which are the main force of the colloquium. But here are a few individual views.

Take Professor Louis Lliboutry, for example, a world-renowned glaciologist with a position at the University of Grenoble and a laboratory supported by the Centre National de la Recherche Scientifique, (the major basic science organization in France). His subject is not one of the current fashions; glaciology does not have much impact on technology and the French economy; the laboratory is away from the centre of power in Paris.

Yet Grenoble is an excellent place to do glaciology, says Lliboutry — he can be at Mont Blance in an hour by car - but he felt this subject had been neglected by CNRS. "Our budget increases have not kept up with the cost of living", he said: and whilethe real money had shrunk, CNRS had shifted more and more resources out of the general budget and into special pro-grammes, the Actions Thématiques Programmées (ATP). So in the past if your research did not fall within the framework of an ATP, you were neglected. In fact the exercise of raising cash had become largely one of convincing the relevant committee that your research fell within this or that ATP. For glaciology, one could sometimes make contact with the physics of tectonic plate movement or climatology, for example. "The people in the committees are the most noisy, but not necessarily the most brilliant", said Lliboutry.

There is an alternative now, however, on

the ATPs — whose use increased greatly under Giscard d'Estaing's science minister, Pierre Aigrain. That's that they provide an opportunity for new researchers and new groups to draw on CNRS funds, in competition with established laboratories.

Unfortunately for Lliboutry and other laboratory directors who share his views, the emphasis on ATP increases slightly under the new government: CNRS funds for ATP rise 41.5 per cent this year compared with last. General programme support in CNRS will also rise, but less strongly: 36.8 per cent. Funds for big equipment (costing more than FF 100,000-£10,000) increase least: 10.5 per cent.

However, another of Liboutry's worries may be dealt with by the research "law", the "Loi de programmation et d'orientation" (see page 289) which is currently receiving the attention of the economic and social committee of the French Assembly. The problem is flexibility in the use of funds.

"We lab directors have no free money" says Lliboutry. This is the real obstacle to good research — what Lliboutry calls "the Kafkaesque rules for finance". These are imposed by the Ministry of Finance, but also by CNRS itself. For example, suppose Lliboutry asked for FF 100,000 for glaciology equipment and another FF 100,000 for general research expenses, for a project within some ATP; and suppose that ATP committee awarded only the latter FF 100,000 but ignored the equipment request. Then Lliboutry could not divide the sum 50:50 as he might wish.

This might change — at least in so far as the Ministry of Finance is concerned — if the *loi* is passed by the assembly, for the law will loosen constraints of this kind on CNRS. How far CNRS will choose to devolve this new freedom to its laboratory directors, and how far it will want to keep control through its central committees, is undecided, however.

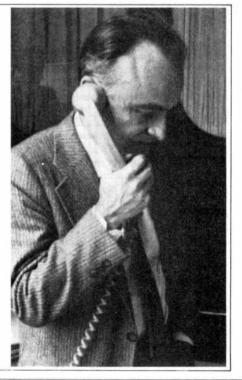
Jean-Jacques Payan, the recently appointed director-general of CNRS, would in fact be well-advised to devolve this new power (if, of course, the Assembly approves it). Lliboutry's complaints are far from unique; they represent a widely-felt unease in the regions of France that the control of science has always been too hierarchical, too Paris-based, and that the minister for science, Jean-Pierre Chevènement, while paying lip service to "democracy", has in fact such well-formed plans that Paris control will continue as strongly as before.

"We need a local esprit d'enterprise in small science, with the freedom and the power to make judgments locally", Lliboutry suggests, with decisions up to a certain level taken by local laboratory councils. Many speeches and papers at the Assizes Regionales and the National Colloquium made the same or similar points. The loi, it seems, will set up regional science committees charged with esta-

Colloquium into law

PIERRE PAPON (right), professor of physics in the University of Paris, is a prominent member of the science minister's cabinet. Papon was responsible for assessing the sometimes diffuse results of the National Colloquium on Science and Technology, and putting these conclusions into effect in the new law for science (see page 289).

Papon identifies four principal effects of the colloquium on the law: on regional development (regional councils for science were proposed at the colloquium, and have found their way into the law); the principle of education through and by research — which is to say that the grands organismes should have a role in education as well as the universities; other improvements in the training of scientists; and the recognition of research as a career so a researcher could expect similar conditions of employment in different organizations, to allow more mobility.





The CEA research centre at Grenôble, on the borders of the French Alps. The dome of the Institut Laue Langevin reactor building can also be seen on the left. The area includes many important laboratories and represents the largest concentration of physical science in France outside the Paris region.

blishing regional science policies and spending a certain amount of money, but it is unlikely that these bodies will have much effect on basic science.

The opposite concern — expressed at the other end of affairs, in Paris - is that there is a degree of rigidity in the system of science management, caused in part by the existence of laboratories whose directions are difficult to shift. Although in principle research posts with the big three research organizations - CNRS, INSERM (for medical science), and INRA (for agriculture) - are contractual, and assessments of performance of staff at laboratories are made regularly, laboratories are very rarely closed down and people almost never fired. Professor Charles Thiebault, who resigned last year as president of CNRS over a conflict with science minister Chevenement, says that of the 1,200 or so CNRS laboratories (one third of all university laboratories), perhaps one or two were closed each year and staff were moved on to new work. Moreover, a new contract for all research workers, contained in the loi, will give researchers even more security, matching that of civil servants.

Professor Michel Petit, director of INAG, which is effectively a wing of CNRS concerned with managing astronomy and geophysics, points to another stiffness in the joints: applicants for CNRS posts specify which laboratory they wish to join. So although assessment is made nationally, keeping standards up, students tend to return to their old professor's laboratory, reducing mobility.

These are directors' complaints, however. Down at the grass-roots, some rather more anarchic notes are being struck. At the Strasbourg Assize Regionale, for example, there was a strong call for laboratory directors' posts to be made fixed term — perhaps four years — to stop one man gaining lifetime control over a sector of research, and allow a few new

flowers to bloom occasionally.

Even at the Institut Pasteur in Paris, a world-renowned centre for microbiology and molecular genetics, there is a degree of revolt. Laboratory directors should be continuously assessed, some of the younger researchers say. There is a tendency for group leaders and laboratory directors in France to stop doing research and become full-time politicians, they complain - quite unlike the United States, where group leaders might spend time rooting for money but do not neglect the life of their groups. Jacques Monod, the famous director of the Pasteur in the late 1960s and early 1970s, is said to have been an exception. Certainly François Gros, the previous director of the Pasteur, has taken the political path: he is now science adviser to the Prime Minister, Pierre Mauroy.

The myth of the "savant", the wise man to whom all possible respect is due, is also too strong in France for the health of science, young researchers say. Strangely for the country which once slaughtered its aristocracy, there is an almost servile respect for these intellectual aristocrats.

According to Professor Bernard Jacrot — himself a potential savant, who directs the neutron scattering outstation of the European Molecular Biology Laboratory at the Institut Laue Langevin, Grenoble — the problem starts in the secondary schools. "As soon as a pupil tries to be individual, he is stamped on" he says; and this reflects itself in the structure of the French laboratory, which is very hierarchical "leaving little room for originality".

Thus there is a need to give younger researchers their head, perhaps against the will of the all-imposing director, and this is a theme which the powerful French scientific trade unions have taken up with enthusiasm. But there is a difference between flattening the pyramid a little, and turning it upside down.

However, one of the clearest democratic

Big physics

THE Commissariat à l'Energie Atomique (CEA) has a civil research budget for 1982 of FF5,260 million, exceeded only by that of the Centre National de la Recherche Scientifique. The budget is high not so much because the CEA supports a lot of researchers (nearly 9,000 at CNRS, only 900 in fundamental research at CEA), but because the bulk of CEA research is in the applications and development of nuclear energy. The fundamental science is also expensive - reactor physics, accelerator physics, nuclear fusion and high energy astronomy. However, CEA researchers also work in condensed matter and materials science and chemistry and biology (using isotopes). The fundamental research budget of the CEA in 1982 is FF 1,292 million.

The CEA works in strict liaison with the other grands organismes on many joint programmes (such as the nearly-completed nuclear accelerator GANIL, in conjunction with IN₂P₃) and with international organizations such as the European Centre for Nuclear Research (CERN) and the Institut Laue Langevin. Its principal research centres are at Saclay, Caen, Fontenay-aux-Roes, Grenôble, and Cadarache.

indications of the wishes of basic scientists has come from an internal survey conducted by CNRS. Professor Guy Aubert, director of the Service National des Champs Intenses, was charged with collecting the contributions of CNRS staff to Assizes Regionales and related meeting throughout France. Five main themes emerged, said Aubert.

First, people were worried that Mitterrand's and Chevenement's committment to regionalism would mean that peer assessments and appointments would be made and decided regionally. This was thought to be a bad thing, because a critical mass of assessors could not be reached and local savants might become even more influential.

Second, there was strong pressure for more "democracy" in the laboratory — the banner which the unions are flying. There should certainly be consultation through a laboratory council, including administrators and technicians, over major developments. And there should be elections "even, and perhaps especially, of the laboratory director" (Aubert sums up).

Third, it was felt that there was more room for research in the social sciences — not just more money for the field, but a re-orientation of priorities towards current political, economic, and social concerns in France. This view is certainly shared by Chevènement, as the resignation of Charles Thiebault (mentioned above) as CNRS president was the result of the

minister's efforts to force a social scientist of exactly this persuasion onto CNRS, as social sciences director.

Fourth, there was much puzzlement over the role and relationship of all the big organizations which control and fund science in France. "It seems to people in the laboratories that these things are not clear" says Aubert. It is possible to obtain money for a given subject from many different sources, under different rules, and career requirements under different heads for essentially the same work can be quite distinct. This leads to a confusion of careers and priorities, and a reinforcement of the feeling that policy is decided behind closed doors according to unknown rules. Chevènement has said he seeks "transparency".

The situation seems to be most extreme for the medical sciences, and basic sciences bordering on medicine — going as far as molecular biology. Here there are the CNRS, whose major sector is life sciences; INSERM, the Institut National de la Santé et de la Recherche Médicale; the Mission de la Recherche of the Ministry of Universities; the universities themselves; and the teaching hospitals. Dr Jean Dausset, for example, is at the Hôpital St Louis in north Paris. He is also head of an

INSERM unit. And co director of a CNRS laboratory. Or take Professor Pierre Chambon. He is a professor at the Université Louis Pasteur in Strasbourg, and head of an INSERM unit and director of a CNRS laboratory, under the same roof. It is good to know that the new director of INSERM, Professor Philippe Lazar, and the director of life sciences at CNRS, Professor Roger Monnier, aim to resolve the issue.

The fifth and final concern detected by Aubert's review of CNRS papers to the colloquium and its tributaries was over the matter of contracts. As one foreign observer and francophile put it, the urge for security is extreme in France (so the trauma of two million unemployed can be imagined). So unions have been pressing, essentially, for tenure, and a strict demarcation of jobs; conditions of work like those of civil servants (the goal, it seems, of every Frenchman and woman); and an "open" assessment of researchers.

Some of these demands the unions will achieve in the "loi"; others they will not. But the unions are pressing harder on this left-wing government than they did on the previous right wing one, much to the socialists' embarrassment; and what the result will be is anyone's guess.

strengthen existing ones.

It is perhaps unfair to make selections, but some of the outstanding regional centres are Grenoble and Lyons (for physics), Strasbourg (molecular genetics), Toulouse (biotechnology and space science) and Marseilles (immunology). The unique technological University of Compiègne — it can probably be counted just outside the Paris region — is also outstanding in enzymology, particularly as it relates to biotechnology.

The largest sector of the CNRS budget for 1982 goes to life sciences (25.5 per cent), which looms larger than it might because some of the heavier expenses of big science are borne by or shared with the Commissariat à l'Energie Atomique (for nuclear physics) or the Centre National d'Etudes Spatiales (for satellite-borne astronomy). Even so, life sciences are also supported by the Institut National de la Santé et de la Recherche Médicale — whose budget exceeds that of the life sciences sector of CNRS though employing fewer researchers.

Other sectors of CNRS are chemistry (15.7 per cent of the 1982 budget), nuclear and particle physics (12.9 per cent), earth, atmospheric, ocean and space sciences (11.8 per cent), mathematics and basic physics (11.7 per cent), social sciences (8 per cent), physical sciences for engineering (7.9 per cent), humanities (5.5 per cent), and interdisciplinary programmes (1 per cent).

CNRS is in fact organized as a "group" of three bodies: CNRS proper, plus INAG and IN₂P₃. The latter are the Institut Nationale d'Astronomie et de Géophysique, and the Institut National de Physique Nucléaire et de Physique des Particules, and they manage the parts of the total CNRS budget concerned with those fields. INAG and IN₂P₃ are loosely controlled by the CNRS directorate, which selects the INAG and IN₂P₃ directors (from lists of "at least two" names proposed by these bodies) and channels funds to the two

CNRS — the core of research

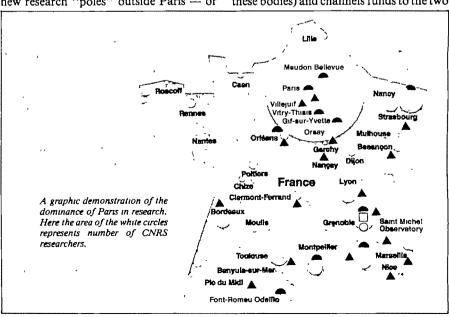
THE Centre Nationale de la Recherche Scientifique (CNRS) has FF 6,000 million to spend this year — about £600 million, including salaries. CNRS attracts and controls the cream of most basic science in France — with the exception of mathematics, which has a very strong French historical tradition and leads a more independent life. With that exception, the goal of most aspiring basic scientists in France is to join a CNRS group or laboratory — a goal which has become even more attractive now, since the new government has increased scientific recruitment to CNRS and its junior partners by 50 per cent, and now that CNRS scientists are promised essentially civil service security, pensions and other rights.

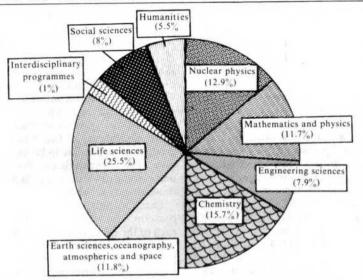
Entry to CNRS is by examination and interview. In 1980, for example, around 3,000 young scientists applied to join CNRS. Most of the 3,000 had just completed a troisième cycle (something between a master's and a PhD degree) at a university; 425 of them were selected as attachés on a four-year trial, after which, according to the 3 per cent recruitment then in force, some two out of three might have expected to receive a full-time appointment as chargé de recherche. With 4.5 per cent recruitment, possibly all of this bunch will be lucky

CNRS now employs 23,320 people, of whom 8,970 are researchers, distributed in more than 1,200 laboratories. More than 150 laboratories are fully controlled by

CNRS (*laboratoires propres*), and another 250 shared with another organization, often a university (*laboratoires associés*).

The laboratories are distributed all around France, but by far the largest single geographical group is formed by Paris and its immediate locality — something which simply reflects the unusual dominance of the capital city of France over the rest of the country, in all sectors of life. However, CNRS has set up regional administrations, with a certain degree of power, and the new government is making an effort to identify new research "poles" outside Paris — or





How the 1982 CNRS budget is being divided up. Life sciences get the lion's share. "Big science" is not as prominent as might be expected as budgets are shared in these subjects with CEA and CNES.

bodies. This year INAG receives FF 115 million, IN₂P₃ FF 576 million from within the CNRS budget. The structure is said to make the management of "big science" easier; certainly, battles over funds for expensive equipment are fought at a higher level in the CNRS hierarchy than might otherwise be the case.

The total CNRS group budget of FF 6,000 million represents an increase of 25

per cent over last year, a general increase which hides even larger rises in some sectors. General programme support and small laboratory expenses has risen 36 per cent from around FF750 million to over FF 1,000 million. Salaries take the biggest single chunk: FF 3,900 million. Increases over 1981 are smallest for large equipment and computers (just over 10 per cent). Against these increases weigh the current

inflation figure of 14 per cent; the increase in the dollar against the franc (up 30 per cent over 1981); and the freeze on FF 150 million of CNRS funds (its share of the 25 per cent new money freeze — see first story).

New posts have also been created: 447 researchers and 193 engineers, technicians and administrators.

The new funds are not distributed equally among all research sectors supported by CNRS. The biggest fractional rise goes to an interdisciplinary programme of solar energy and primary materials (PIRSEM), which will get FF 10 million, over three times as much money as its predecessor PIRDES.

Besides that exceptional case, applied physics (physical sciences for the engineer) is most favoured: it receives a 40 per cent rise. Life science gets 38 per cent, earth science 36 per cent. Life science gets the biggest share of the new research posts (127) but then it also has the biggest share (2,500) of the total research staff of CNRS. The budget increases will be used for four principal purposes. First, to re-equip laboratories to "bring their means back to an internationally competitive standard". General laboratory spending suffered a severe decline between 1972 and 1980, say CNRS staff. The previous science minister, Pierre Aigrain, won a substantial increase in the general science budget in 1981 (8 per

Pressing problems for Payan

THE major issue facing the CNRS director-general, Jean-Jacques Payan, and president, Claude Fréjacques, is the reorganization of CNRS — to which the new government and CNRS directorate are committed.

At present, CNRS is structured around a remarkable scientific parliament called the Comité National, which consists of about 1,000 people, roughly two-thirds of them elected every four years from CNRS laboratories and groups and onethird nominated by the government from among scientists, industry and the administration. Researchers, engineers, technicians and administrators are all represented. The Comité is divided into 41 sections according to discipline. In spring these sections advise on individual careers and promotions, and in autumn they consider the prospects of groups and laboratories.

This advice passes to the eight scientific directors of CNRS, who are usually career scientists who spend a period of 4-8 years on part-time leave from their posts to direct a sector of CNRS activity; and then to the "double head" of CNRS, the director-general and president. Ultimately, major decisions are taken by the governing body of CNRS, the government-nominated Conseil of which Fréjacques is president.

To those interested in increasing "democracy" in French science — which means effectively reducing the power and influence of the scientific directors of CNRS and, at the local level, of laboratory directors — the Comité National plays a central role. The scientific trade unions would like to increase their control and influence over



Payan (left) and Fréjacques face the unions

the parliament. Commensurately, it is felt that the powers of the parliament itself should be increased.

A struggle is taking place between these unions and the directorship of CNRS. The unions demand that nobody be elected to the *Comité National* unless he or she is accredited by a union — which represent mostly technicians, engineers and some younger researchers. Payan has sent a letter to laboratory directors to warn them of the threat and ask for their advice. One senior scientist cabled Payan to say he would resign all his CNRS

functions instantly if the unions were granted such power — and his reaction may not be untypical.

Professor Payan himself is shocked by the union militancy. He said recently that the thorough-going dialogue now under way in CNRS touched all levels, and that CNRS old hands had not experienced such a thing for a long time [since 1968, perhaps]. But, said Payan, whereas the union position against the previous rightwing government had understandably been "the most extreme possible", now was the time for a more comprising attitude. Was the socialist government not, after all, on the same side?

A second important — but less pressing — issue facing Payan concerns the relationship between CNRS and the universities. Under the previous government, CNRS was under the authority of the Ministry of the Universities, but now it is controlled by the Ministry of Research and Technology. Since CNRS already creams off some of the best scientists from education, this shift has led the universities to fear a deepening of the class divisions between university and CNRS laboratories.

This fear is further increased by plans to give CNRS a role in the education of researchers. But, says Payan, a break between the universities and CNRS would be a bad thing. CNRS will cooperate with the universities, he says — but, on the other hand, the universities have a long way to go . . .

cent in real terms, he claims), and this year that trend is accelerated to 25 per cent in real terms.

Second, the aim is to bring to fruition certain plans for large machines — the heavy ion nuclear accelerator (GANIL) and a French dedicated 800 MeV synchrotron radiation source, Super-ACO, at Orsay (in collaboration with the Commissariat à l'Energie Atomique, the Ministry of Universities, and the Université Paris-Sud). Increased support will be given for the reactor Orphée and the trinational high flux neutron beam reactor at the Institut Laue Langevin in Grenoble.

Third, new themes of CNRS support are to be established, particularly through the special programmes called Actions Thematiques Programmées (ATP) under which CNRS laboratories and groups compete for extra money available for work directed to particular objectives — which are becoming increasingly technological. This year ATP cash has crept up to nearly 10 per cent of the research money otherwise available to CNRS laboratories.

Finally, FF 8 million have been set aside for a programme of so-called "young groups", which is designed to give younger researchers a chance to create their own groups earlier than would otherwise be the case. The programme also gives CNRS the chance to go head-hunting in some of the smaller universities for new talent. The name "young groups" may be a bit misleading, however: the eight "young" group leaders recently selected by the life sciences sector of CNRS are all about 40 years old. "We hope we can bring the age down somewhat next year" said a CNRS spokesman.

Medicine hot, agriculture cool

CONTRASTED with the scale of the CNRS. the institutions which support health, medical and agricultural research may seem small, but they have a significant impact on research. INSERM, the Institut National de la Santé et de la Recherche Medicale, supports 1,500 researchers (compared with 2,500 in the life sciences sector of CNRS alone). Even so, this is over a third of French biologists, and the means at INSERM's disposal are not negligible: over FF 1,000 million this year. Since the CNRS life science budget is just under FF 1,000 million for nearly twice as many researchers, INSERM scientists can count themselves rich.

INRA, the Institut National de la Recherche Agronomique, is also substantial — although until recently it had appeared to have been neglected by the new government. INRA has 200 researchers and a 1982 budget of FF 1,400 million. INRA is unusual in being highly decentralized, as befits an agricultural agency, with laboratories all over France and also in Corsica and Guadaloupe.

INRA politics, for the moment, are relatively quiet: INRA's director-general, Jacques Poly, has not yet felt obliged to resign over some difficulty or other with the new minister of science — unlike his former colleagues at CNRS and INSERM, who did just that. But perhaps that is because the ministry has hitherto paid little attention to agriculture. Earlier this month, however, the establishment was announced of a new department at the ministry of research and technology, to be concerned with agriculture and food.

Chevenement appears to have noticed that the agroindustry employs 3.5 million workers, and that its activities concern every consumer in the country.

The new department will assess the present state of research and development in this area in France; define priority research areas; stimulate relevant research in small industries and businesses; extend the links between INRA and industry; establish the needs for training and recruitment; and take account of the needs of developing countries.

What Professor Poly makes of this has yet to be seen, but meanwhile the new director-general of INSERM, Professor



Lazar - back to the watering can?

Philippe Lazar, is settling in after his predecessor's resignation. That gentleman, Professor Philippe Laudat, was very oblique about his reasons for resigning, but the speculation is that he feared that his efforts to concentrate INSERM funds on the best groups, so avoiding what he described as "an even sprinkling" of money regardless of quality, would be reversed by the new government. As what Laudat identified as "best" often meant groups working in molecular biology, INSERM researchers in that area are worried.

For Professor Lazar, if anybody, would be the one to apply the politics of Chevenement to INSERM: he was rapporteur for the National Colloquium of Science and Technology in January. So what does he plan to do?

Laudat certainly had trouble with the scientific trade unions, with his determination, as he put it, to open up INSERM research; and Lazar will clearly be more sympathetic to union views. Lazar sees no reason to be so selective with basic research money as was Laudat, because research funds for all basic science are guaranteed to rise 13 per cent in real terms (according to Chevènement's forthcoming law).

"We should try to mobilize almost all research units" says Lazar, "and be a little more confident in the ability of all groups". It is possible to be too elitist, he says. How is one to detect which research unit is going to be successful, as opposed to those already at the top?

Lazar intends to apply a policy of a posteriori, rather than a priori assessment: each of the INSERM units will be expected to present a programme every two or three years in which it expresses its needs; INSERM will then respond. This will save laboratory directors time in their constant efforts to raise money. Each unit will be assessed at this one time by INSERM's eight specialized committees and scientific council, which will give advice on the closing of certain units and the opening of others. But the specialized committees should "follow each laboratory more closely and interfere more".

It should be easier than at present to change the directors of laboratories, says Lazar. As thing stand, a director is appointed for six years — after which he or she is assessed and, almost without fail, reappointed. "The general rule should be that a director should change", says Lazar.

Lazar also feels that the links between the INSERM administration and its scientists are too weak, and he would like to make use of the 225 committee members more than the present twice a year; and he wants to try to improve INSERM's links with the outside world, including CNRS, the Institut Pasteur, the universities, and institutions beyond France.

CNRS and INSERM

Lazar would like particularly to rationalize the overlapping relationship with CNRS, a desire shared by CNRS director of life sciences, Professor Roger Monnier. Sometimes both INSERM and CNRS support the same laboratory in different ways, and neither knows what the other pays. Lazar favours joint committees set up between CNRS and INSERM.

Of molecular biologists relying on INSERM grants, Lazar says "they may be afraid but I am not". Molecular biology will receive "at least 13 per cent" in real budget rises in coming years — as a basic science - and in so far as the work is linked to biotechnology, nearer 30 per cent. For Lazar will countenance certain programme mobilisateurs which will receive more than the average attention. Biotechnology will be one; also the social sciences of health care. Clinical research, generally thought to be weak in France, will also receive special attention, by "opening it up to other research". Lazar intends to hold a meeting to illuminate that issue.

gearing up Biotechnology -

BIOTECHNOLOGY - including the most basic molecular biology - is to be one of the principal axes of M. Chevenement's French technological revolution. Moreover it is the first of his ministry's programmes to get off the ground, because its main points are identical to those followed by the previous government. This is not least because the biotechnology adviser to the previous government now spearheads Chevènement's biotechnology department at the ministry. He is Professor Pierre Douzou, a well-known microbiologist at the University of Paris. The only obvious change from the earlier policy seems to be the fivefold budget increase.

The main weaknesses in French biotechnology have been identified as a woeful lack of microbiologists, a poor system for training genetic engineers, too little work on plant cell biology and weak links between the worlds of research and industry. The genetics of nitrogen fixation is also thought to be poorly represented in France; and lastly, the French pharmaceutical industry is particularly weak in the antibiotic market, and so has little experience in the sophisticated fermentation technologies that antibiotic production requires. On the other hand,

France can boast some world-class groups in molecular biology, particularly at the Institut Pasteur and the University of Strasbourg. The lack of microbiologists is perhaps surprising in the country of Louis Pasteur, but just as in other countries, French biologists have followed the trend towards concentrating on "laboratory" species such as Escherichia coli. To correct the trend, CNRS is planning to set up new groups or laboratories working with species such as Pseudomonas or Actinomyces, and, they say, they are ready to import the right group leaders from abroad. French universities are also preparing to establish professorships in microbiology. Also, plans are almost complete at CNRS to establish a new laboratory of plant genetics in Strasbourg, at a cost of around FF40-50 million, which will employ 100 scientists.

At the Institut Pasteur in Paris construction is going ahead on a new building devoted to biotechnology, to open in 1984-85, according to a decision taken by the previous government. Among other things the building will provide facilities for 5-10 litre fermentation trials and will have a laboratory for the development of

hybridomas.



Science and the scientific are chic right now in France: witness this advertisement for DNA cream from a French magazine.

At present the strong poles of CNRS biology with an impact on biotechnology are at Strasbourg (basic molecular biology and plant science), the Institut Pasteur (all fields), Toulouse (selected by Aigrain to be a special centre for biotechnology), Marseilles (laboratories of immunology

The Pasteur leads the way

THE Institut Pasteur, founded by Louis Pasteur in 1888, three years after his development of an antirabies vaccine, became and remains one of the most important biological laboratories in France; and now, it is also taking a leading role in the development of French biotechnology.

This is happening both at the political and practical level. François Gros, the previous director, has taken a deep interest in biotechnology, and in his last three years as director (he resigned at the end of last year) had begun a slight shift of the Pasteur away from human and animal pathogens (its traditional interest) towards microorganisms of potential economic importance. Now he has become science adviser to the Prime Minister, Pierre Mauroy, where he has considerable influence. Moreover, the National Colloquium on Science and Technology, the great conference of scientists and public that has been one of Chevènement's most imaginative achievements, was Gros's idea. Gros organized and chaired the colloquium, so endearing himself to the new minister of science as well as the Prime Minister.

On the practical side, the Pasteur is now seen in government circles as an excellent model of the combination of fundamental research with industrial and medical development, a kind of mix of priorities which can be traced directly back to the inspiration of Pasteur. The Pasteur has a hospital. It has research which reaches the most fundamental levels of biology (three of the six French Nobel Prizes since the war went to the Pasteur). And it has three distinct ways in which it tries to exploit its research.

There is Institut Pasteur Production, which has a first call on potentially profitable discoveries at the Pasteur; Groupement Génie Génétique (G3), which seeks directly to develop profitable ideas, which it would then license to industry; and a highly differentiated and active system for linking Pasteur staff with industry, making a profit for the Pasteur and aiding the biotechnology industry at the same time.

The latest effort to be announced is a massive trial of a new vaccine against hepatitis B, produced by Institut Pasteur Production, on 200,000 Senegalese children, in conjunction with the Institut Pasteur (Dakar) which is one of the 26 institutions around the world linked to the Pasteur (Paris). Nine out of ten adult Senegalese are infected with the virus, it is estimated. If the trial is successful, there is a big world market for the vaccine.

The Pasteur is also gearing up to train the bioengineers that France will need. Special training will be given to engineers emerging from certain grandes écoles. Links have been established so far with Polytechnique, Centrale, Mines, and Ecole Nationale Supérieure de Physique et Chimie de Paris.

The original Institut Pasteur building of 1888. Pasteur's tomb (see cover) is on the site.



and bacterial chemistry), Compiègne (enzymology and enzyme fixation) and to a lesser extent, Lyons and Montpellier.

Douzou outlines the principal objectives of the ministry as first, to improve training and education in the field; second, to transfer information more efficiently from the laboratories to industry; and third to establish "a multitude of sites" in France as small technology centres, which would embody perhaps four or five researchers, several engineers, and technicians, each working on some pilot scheme. These technology centres should be encouraged in industry as well as in the public sector such as CNRS and INSERM, says Douzou.

In that public sector, Chevènement has said that such laboratories would be judged by the patents achieved, by licences sold to industry and by their general impact on the industrial sector rather than by their scientific productivity *per se*.

However, development work in biotechnology can be very expensive, and France is certainly willing to consider international agreements and programmes at that level. They should be bi- or trilateral and specific, however, rather than conducted through an intermediary like the European Commission in Brussels.

Industry cloning

FRENCH industry boasts a handful of main actors in genetic engineering. They are Transgène, a venture company with a small amount of government support based in Strasbourg; Genetica, a genetic engineering wing of the chemicals giant, Rhône-Poulenc; G3, a group based at the Institut Pasteur in Paris and supported by government money through CNRS, INSERM and INRA and (51 per cent) by the Pasteur itself; and the nationized oil company Elf Aquitaine. Elf is to set up a FF100 million biotechnology laboratory in Toulouse, and through pharmaceutical subsidiary Sanofi it has a 51 per cent share in Institut Pasteur Production, the company with first claim to the development rights of Pasteur Foundation discoveries.

None of these companies except Institut Pasteur Production (which is a an older company with a strong interest in vaccine production) yet has a marketed product, and all are keeping their plans fairly close to their chests. But Transgène, at least, is known to have commissioned reports on the microbial extraction of minerals from sea water and to be considering a special effort on plant cells - stimulated by CNRS plans to make Strasbourg a focus of plant genetics. Transgène's director, Jean-Pierre le Coq, is also looking closely at what the new biology can do in traditional biological industries such as food processing - where, he says, the field is huge and the competition less.

Telecommunications — late start

FIVE years ago, the French ministry of telecommunications, PTT, launched an ambitious plan to improve the services offered by the public telephone network. Since then, massive investment has increased the number of telephone lines from 7 million to 16 million. When he presented his budget to the assembly earlier this year, Louis Mexandeau, the new PTT minister, indicated that he wishes to continue the modernization albeit at a slightly slower rate. He is looking for a further FF 25,000 million a year for the next five years to give a telephone to everyone who wants one by 1985.

As PTT admits, the success of the modernization so far is partly due to the initial small size and poor state of the telephone network. France has been able to opt for the latest technology and avoid the problems inherent in modernizing an out-of-date, yet extensive, system. Hence, for example, France has been able to move over to fully electronic exchanges move rapidly than some of its European neighbours. The plan now is to digitize the network fully by 1990.

To achieve its goals, however, PTT has had to lean heavily on industry. Although the result has been to increase the international competitiveness of the French telecommunications industry, there have been problems along the way. Pierre Aigrain, former science minister who has now returned to his old haunt Thomson CSF as research director, mentions the difficulties caused three years ago when PTT changed suddenly from installing partially to fully electronic exchanges.

The switch meant that Thomson had to reduce its workforce, a task-it has still not managed to complete. Thomson was also at a disadvantage because, unlike its competitor, CIT-Alcatel, it did not receive state help to develop electronic exchanges. PTT supported development in its own research centre, the Centre National d'Etudes des Telecommunications (CNET), and then licensed the product for manufacture by CIT. The CIT product works well, but Thomson is still having software problems.

(The PTT supports most of its research on electronics at the CNET laboratories in Grenoble, which, like the laboratories of CNRS, have been asked to step up the number of researchers employed by 4.5 per cent a year. The ministry of research and the PTT now want the Grenoble laboratories to develop into a major centre that will drive not only telecommunications but also the broader electronics industry.)

Switching to optics

The French have also been fortunate in their choice of technology. By choosing a time division switching electronic exchange, they have suffered fewer technical problems than say Britain with its choice of System X. But installing

electronic exchanges is just the first step in PTT's vision of transforming the network into a modern system for digital rather than analogue transmission.

Like its European neighbours, it is nibbling vigorously at the bait of optical fibres. Thus a 12 km optical link has been installed between two telephone exchanges in Paris and two more links are planned.

Optical fibres are still too expensive for widespread introduction, largely because the signal has to be boosted at regular intervals of about 30 kilometres. The PTT will be waiting until the price comes down to about FF1 per kilometre. But Saint-Gobain and Thomson CSF seem confident that the market will expand soon. Recently, they formed a joint company, Fibres Optiques Industries, with Corning Glass, to manufacture 10,000 km of optical fibres a year, half planned for export.

Optical fibres are also being connected to 2,000 households in Biarritz in the south of France for an experiment, beginning in 1983, that will give each household access to new telecommunications services such as videophone, videotex and cable television, via a telephone linked to an ordinary television screen. A further experiment to bring cable television via optical fibres to the people of Lille is also planned. The aim is to test the usefulness of new technologies and the information they bring to individuals and businesses before launching into full-scale development.

Planners seem particularly keen to bring new telecommunications technology to the business community. In 1983, French companies will be able to make use of the services of a satellite more or less devoted to business communications when Telecom 1, a satellite being built with PTT backing under the direction of the French space agency, CNES, is launched. In addition to providing business links, Telecom 1 will free France from the expense of leasing channels on Intelsat satellites for telecommunications links with its overseas territories.

The Mitterrand government clearly shares the view of its predecessors that modernization of the network is essential. But where the old and the new policies may part company is in a strategy for widespread introduction of télématique — the uniquely French word for the information systems created by linking computers via telephone lines — into homes. The new government is sceptical of the economics of the Giscardian plan to give every home a télématique terminal. And Jacques Dondoux, the new director of telecommunications, has made a concession to public anxieties about the social impact of télématique by agreeing to set up a users' group to discuss the issues.

The previous plan to install and experimental electronic telephone directory into every home in Ille et Vilaine in Brittany has

thus been modified to give householders the right to refusal. Householders in Velizy, a small town near Paris, are also to be allowed to choose whether to participate in an experiment to monitor the use of Teletel, the French public viewdata system. The results of both these experiments, which began last year, will be fed into a national debate on the new technology.

PTT plans to finance its future investment programme in much the same way as it has in the past — from earnings and money borrowed abroad. As the network grew, the proportion of investment raised from receipts grew from 43 per cent in 1977 to 74 per cent in 1980. The hope is that three quarters will come from cash flow.

Thus the pressure on the public purse should continue to be minimal. But the Mitterrand government, in its eagerness to offer the benefits of new telecommunications technology to everyone that wants it, has already suggested that PTT offer special tariffs to certain groups, for example the elderly. If it sticks to its guns, then PTT will be looking for public money to make up the deficit.

Judy Redfearn

Microcomputers for all

WHILE other countries agonize over the social impact of new information technologies, France is preparing to take the bull by the horns in the form of a new centre set up last October with an annual budget of FF 100 million. The Centre Mondial, as it is known, is the brainchild of Jean-Jacques Servan Schrieber, author of the book *The World Challenge*, who believes that microcomputers can wipe out social inequality by bringing information to all. Last week, the Centre Mondial held its first assembly.

Professor Nicholas Negroponte, professor of computer graphics at Massachusetts Institute of Technology is managing director. He will be supported by a board consisting of 12 French cabinet ministers and a group of French and foreign scientists. Around 50 researchers will be appointed this year and a further 50 by the end of 1983.

The idea, however, is not simply to get computer scientists to design micro-

computers. The public must also be involved in testing new and existing information technologies. Hence the centre will be establishing offices where passers-by can test out various wares. The ultimate aim is to feed public reaction into the design of a powerful microcomputer, costing no more than £100, for personal use.

In the meantime, the centre will study social questions such as the human-computer interface and the value of information technologies to members of different cultures. Two projects have begun. One, to bring microcomputers to a quarter of Marseilles in collaboration with the University of Aix at Luminy, will test the French response to the new technologies. But the other, to train young people from Senegal in the use of microcomputers, will be used to assess the usefulness of the new technologies to those living in developing countries.

Judy Redfearn

National trend in space

THE enthusiasm of the Mitterrand government for science and technology has not been lost on the Centre National d'Etudes Spatiales (CNES), the French national space agency. CNES has been promised 18.7 per cent more money, in real terms, in 1982 than in 1981. The lion's share of the FF 3,000 million budget for this year, which makes CNES the largest national space agency in Europe, comes from the research ministry. But other government ministries which must contribute towards development of services they wish to use have also stepped up their payments. These include the ministry of defence, which wishes to launch satellites on Ariane, and PTT (the telecommunications ministry) which is contributing towards the cost of Telecom 1, a national telecommunications satellite.

For the time being at least, the new government seems keen to support the space policy of the old. Thus, last October it formally approved CNES's involvement in four new projects of the European Space Agency (ESA) and a second national remote sensing satellite, Spot 2. CNES will have no difficulty spending this year's extra money according to Professor Hubert Curien, the centre's president, largely because Spot 1, the first national remote sensing satellite, is now entering the most costly phase of its development. But if the budget continues to increase over the next five years, as promised, how will it be spent? CNES hopes to persuade its own government and those of its partners in ESA to embark on projects that rely less than in the past on the good will of the United States and the Soviet Union for their success.

Since CNES was created 20 years ago this month, it has followed a policy of multilateral and bilateral cooperation that has benefited both national space industries and the space science community. Hence, membership of ESA has given French engineers the opportunity to participate in the development of their brainchild, Ariane, a project that would have been too costly for national development. And the policy of bilateral cooperation with the Soviet Union as well as the United States and individual European countries has given the French space science community perhaps more opportunities than its counterparts in, say, Britain and Germany.

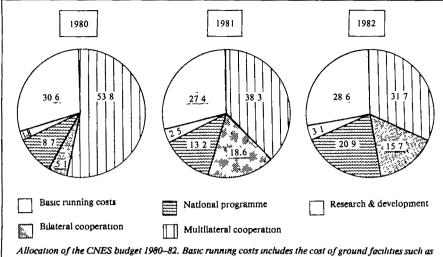
International collaboration, however, has not always run smoothly. CNES still remembers, for example, NASA's decision to withdraw from participation with ESA in the International Solar Polar Mission. Joint projects with the Soviet Union have caused similar frustrations. And the decision of the Soviet space agency to extend its next mission to Venus to include Hally's comet meant that CNES had to abandon plans to send a probe into the atmosphere of Venus.

CNES now wants to shake off the shackles of being the junior partner in collaborations with the two space powers, whilst retaining a strong commitment to Europe on the grounds of necessity. It is following two paths towards its goal. The first involves persuading the French government to support an enlarged national space programme, especially in science. And the second involves persuading the members of ESA to develop an ambitious launcher as a successor to the current series of Arianes.



Hubert Curien — keen to promote ESA as senior partner in bilateral projects

The national space programme centres on the development of applications satellites, five of which will be launched in the next three or four years. These include a telecommunications satellite, Telecom 1, due for launch in July next year; a direct broadcasting satellite, TDF 1, identical to West Germany's "TV-sat"; and two Spot remote sensing satellites for launch in 1984 and 1985. But when these projects come to an end, CNES may well decide that most future research and development in applications, especially telecommunications, should be hived off to national industry which has already developed more expertise in space technology than many of its European counterparts.



Allocation of the CNES budget 1980-82. Basic running costs includes the cost of ground facilities such as the technical centre in Toulouse. The amount spent on multilateral cooperation has decreased as ESA's programme to build Ariane has reached the end of the first development phase. The increase in the proportion spent on bilateral cooperation in 1981 was due to the start of the Venus-Halley mission with the Soviet Union and the Franco-German collaboration on direct broadcasting satellites.

The field could then be open for the first national scientific satellites since 1975. The French space science community, enthused by the possibilities, thrashed out about ten proposals at a meeting last year for wider discussion at the national colloquium on science and technology held in Paris last January.

CNES is studying the feasibility of all the proposals, some of which will eventually be put to ESA. But two, a gamma-ray observatory, Sigma, and a wave altimetry experiment, Poseidon, have been singled out for national development. The reasons for choosing them were their scientific merit and the urgency with which they would need to be developed. The plan is to launch Sigma at the end of 1985 on the first qualification flight of Ariane IV, the latest

Da ili nyet

THE programme to put a French astronaut in space aboard Soyuz-Salyut later this year contributed to CNES's mistrust of bilateral deals when the Soviet space agency refused to release details of the spacecraft for reasons of military secrecy. French engineers then had no option but to guess the specifications for the instruments they are putting on board. In the event, the Soviet space agency allowed French technicians to travel to Moscow for consultation provided that they did not enter the buildings where the spacecraft were being built. But in general, when French engineers ask whether their guesses are correct, the Soviets simply reply "yes" or "no". The result, according to Professor Curien, is "trial and error with rapid convergence". Added to these technical problems is the growing row among French space scientists over the political wisdom of Soviet collaboration. Many are now objecting because of events in Poland and the human rights issue.

Judy Redfearn

version of Ariane to be approved, and to carry the Poseidon experiment on board Spot 2.

CNES now has the task of persuading the French government to find the money for these projects, which could entail almost doubling the space science budget. Financial pressure could be eased, however, if other countries could be persuaded to participate as junior partners. Discussion over collaboration on Sigma has already begun with scientific groups in Italy and Britain.

Thus, with national scientific projects, CNES hopes to regain the power of decision it believes it has lost in many of its international undertakings. Professor Curien would like ESA to follow suit. France had supported moves by Germany at the end of last year to increase ESA's science budget by 20 per cent after 1983. Other members states vetoed the proposition but CNES now hopes that if some of its scientific proposals cannot be afforded within ESA's mandatory science programme, then sufficient member states can be persuaded to participate on a voluntary basis.

Professor Curien believes that ESA too could gain greater control over joint projects with non-European countries if it initiated attractive projects in which, say, US scientists could be invited to participate. His proposal, however, has met with resistance from some members of ESA who fear that US participation could swamp what should still be essentially European projects. The outcome may depend on the findings of a committee of the European Science Foundation.

The strongest evidence of the importance that CNES now attaches to national and European independence in space, however, is to be found in its belief that Europe must build a new generation of launchers that will rival the descendants of the US space shuttle in the 1990s and beyond. At a meeting organized by CNES earlier this year, Yves Sillard, then

director-general, urged representatives of the European space industries to come up with proposals within the next two years. The French idea is to develop an advanced launcher consisting of the first stage of Ariane IV with a powerful cryogenic motor for the second stage. A third stage could be added later. CNES engineers plan to put a proposal along these lines to ESA by early 1984 with the aim of reaching a decision in sufficient time for an operational launcher by 1994.

The French philosophy is to design a flexible system that could launch the future generation of applications and scientific satellites and give Europe independence in new space applications such as material processing. The dilemma is knowing whether Europe will need men in space or whether it should design fully automated systems. CNES engineers are trying to avoid the problem by designing a partly recoverable, automatic system, initially compatible with Ariane IV, that could be converted to manned spaceflight, using the more advanced launcher, when and if the need arises.

Two separate but related projects are under study. The first, Star, a geostationary satellite that would maintain permanent contact between satellites and a ground station by relaying signals between them, may be affordable nationally. The second, Solaris — consisting of one or two Star satellites, an automatic space station in low Earth orbit and an automatic recoverable servicing vehicle - would be costly enough to justify collaboration within ESA. The recoverable vehicle would be of sufficient power to ferry, for example, materials for space processing between earth and the space station. It would also be capable of carrying out routine maintenance and repairs on the space station. Fitted with a suitable boost motor, it could be sent on servicing missions to satellites in geostationary orbit.

The development of both Star and Solaris demand research on space robotics and advanced control systems which CNES and ESA are currently supporting, albeit on a small scale. Ideas for putting men in space centre on developing a suitable recoverable servicing vehicle and space station for the Solaris system and a more powerful launcher. CNES is supporting feasibility studies of a small manned vehicle, Hermes, and a cryogenic motor with up to 100 tonnes of thrust for the second stage of the advanced launcher.

The French government, with its plans for dragging French science and technology to near the top of the international league and with its penchant for national independence through international collaboration, seems set to go ahead with these plans. But the success of CNES's proposals will depend critically on whether other governments, which may prefer to leave the expense of advanced launch systems to NASA, can be persuaded to play ball.

Judy Redfearn

Taking a nuclear power lead

"ONE reactor, one producer, one seller": that is the structure of the French nuclear industry, and according to one observer it is a prime example of how France has managed to mobilize its technical resources towards particular goals. Whether this technocratic centralism will survive a socialist government has yet to be seen, but the nationalization of large sectors of industry, and the grand "mobilization programmes" of the minister for research and technology smack not so much of a change as a clarification of previous government positions.

The reactor is the pressurized water reactor (PWR), built under licence from Westinghouse in the United States; the producer and seller the 5,000-strong company Framatome, now geared up in its pressure vessel and steam generators shops at Le Creusot and Chalon to produce six 1,300-MW reactors a year. Mitterrand did not nationalize Framatome. It was already in effective government control through its sole French customer, Electricité de France (EDF) and its principal collaborator in research, the Commissariat à l'Energie Atomique (CEA).

The main impact on the Framatome programme has been the reduction in the EDF order from a possible nine reactors over the next two years to six, which leaves Framatome with a large surplus capacity and a hunger for export orders. Framatome has sold one reactor a year for export over the past eight years: two to Belgium, both to come into operation this year, two to South Africa (to operate next year), two to Iran (although the orders were cancelled by Khomeni with the plants halfcompleted) and two to Korea. The sale to Korea was a considerable achievement because Korea's previous reactors had been constructed by US companies.

Nucléaire, s'il vous plaît!

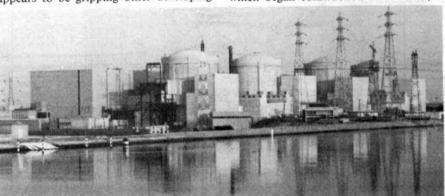
A SLIGHT majority of the French people is in favour of nuclear power, a poll conducted just before the October 1981 energy debate indicated. Some 45 per cent are in favour, compared to 40 per cent against and 15 per cent who don't know, in a sample of over 2,000 people. On the extremes, 16 per cent were "definitely" favourable, and 17 per cent "definitely" unfavourable.

One surprising result which might give the government pause, however, was that 64 per cent were in favour of a referendum on nuclear power — an election promise that was subsequently dropped. Another surprise: nuclear power is more supported on the political left than on the right. Compared with the average 45 per cent in favour, 51 per cent of those who voted communist and 52 per cent of those who voted socialist favour nuclear development.

Framatome has also put in tenders for two reactors for Taiwan and two for Mexico - and if it were not for the world recession would be confident of orders. The dollar rose by nearly 30 per cent against the franc during 1981 (from FF 4.5 to FF 5.8), giving Framatome a distinct price advantage - which is enhanced by the company's ability to spread its overheads over a large national programme. However, Mexico, at least, concerned about its economic situation, is beginning to have doubts about whether it needs the 20 reactors it was planning to build by the year 2000, and the same fear appears to be gripping other developing

Coudray, is that while the Texas reactors are not likely to go critical before 1984 (three to four years late), the first Framatome 1,300-MW reactor — at Paluel — should be connected to the grid early next year. Framatome will thus have taken less than six years to build it, whereas Westinghouse will have taken more than ten years for a similar reactor.

In part, this achievement can be put down to standardization. Framatome has not had to make substantial changes in design in mid-stream to meet changing safety regulations. Apart from the first five reactors, which were fairly variable — Framatome was on its learning curve — there have essentially been only three designs: a group of 16 reactors of 900 MW which began construction in 1974-76; a



Pride and glory reflected - four of France's "production line" PWRs, at Tricastin

states interested in nuclear power. So Framatome may be forced to look to the home market.

In France, Framatome is now building 26 reactors with a total power of 28.6 GW electric, 16 at a nominal 1,300 MW and 10 at 900 MW. Another thirty units, accounting for 21.8 GW, are already in operation. The smaller reactors are effectively the original Westinghouse design, as modified by Framatome; but the 1,300-MW plants are almost completely French, and a new 1,400-MW design, called "N4" and now on offer to EDF, is totally so.

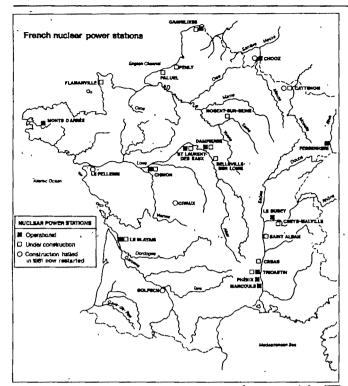
Going it alone

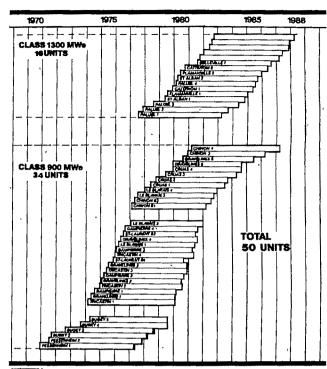
The Westinghouse licence expires this year and then Framatome will be officially on its own - apart from certain research agreements which will continue - but already the sluggishness of the US nuclear programme has given Framatome a commanding lead. According to Framatome's technical director, M. Michel Coudray, the company began work on the 1,300-MW design in 1975. By then Westinghouse had already sold two 1,300-MW systems to a Texas utility, and Framatome believed that it would be able to benefit from Westinghouse experience before putting the final touches to its own 1,300-MW plants. But in the event, construction of the Texas reactors has ground almost to a halt, due to quality control problems on site and concern on the part of the Nuclear Regulatory Commission. The result, said

second group of 12 begun in 1977-81; and the 1,300-MW reactors, begun in 1977 in parallel with the second tranche of 900-MW systems. The result has been that Framatome has been able to place long orders for components, which gives a guarantee of supply, and streamlines production processes. (One danger, however, is that mistakes, once made, are propagated over many systems; see p.301.)

With this powerful production system now established, Framatome would, of course, like a long list of orders; but even under the unbounded nuclear enthusiasm of the previous government, orders came only in occasional handfuls. Furthermore, EDF is now producing 40 per cent of its electricity from nuclear power, and while it aims for 60 per cent nuclear by 1985 (compare Britain's present meagre 11 per cent contribution), there is finally a limit to the proportion of an electric power network that can be driven by nuclear systems. The reason is that national power demand fluctuates by as much as 40 per cent during a working day, and nuclear power stations are not easy to turn on and off: they are said to provide "base-load power". So already, EDF has insisted that Framatome consider the problems set by imposing a ten per cent peak to peak fluctuation on demand.

This so-called "daily load follow and frequency control mode" leads to a number of new technical problems, caused by the high frequency at which the control rods have to be driven in and out (up to





The map shows the sites of the French PWRs, filling many of the suitable sites on France's rivers. The figure on the right illustrates the pace of the PWR construction programme since the early 1970s. The boxes extend from the start of construction to connection to the grid.

1,000 times a day according to some estimates) and by the thermal stress on components as the core heats up and cools.

Nevertheless, Framatome has undertaken an extensive test programme at Cadarache, in conjunction with EDF and the CEA, to discover the behaviour of the reactors under such conditions, and the company believes it is now in a position to make the necessary design changes.

By 1990, French orders may be down to two reactors a year. Outside France, Framatome has sought to sell in many countries — including Britain, whose rejection three years ago of a possible arrangement with Framatome is still a bitter memory in the company. The UK Central Electricity Generating Board was said to be quite enthusiastic about an agreement with France over PWR construction, but the Atomic Energy Authority (AEA) was cool. "Britain could have had an industrial agreement, or a research agreement or both but she decided to go with Big Brother [the United States]", said Coudray recently. "Once again Britain has preferred America to Europe" he said, reflecting a feeling in France that Britain is on all fronts a reluctant European partner.

In the United Kingdom, the chairman of

the UKAEA, Dr Walter Marshall, sees things slightly differently. At the time of the French approach Framatome was stil bound by the terms of the licence with Westinghouse, Marshall points out - sc Britain would have had to "throw ou Westinghouse", with whom negotiations were well advanced, without being able to sign up with Framatome until this year Also Britain would have found it difficul to modify the French design to suit British conditions, for that would have been ar implied criticism of French safety judgements, Marshall argues. Westing house itself, however, was more sanguing about design changes because it could always claim that in the United States it: designs were bound by the rules imposed by the Nuclear Regulatory Commission which might or might not be considered sensible. In France, by contrast, the close knit character of the nuclear establishmen implies that changes are a challenge to the whole system. The Framatome PWR is the world's safest, cheapest and best PWR: sc why change it?

Another, purely commercial reason for rejection of the French deal was that there has been a recognition in Britain that Westinghouse, in the past at least, has been the world's most successful reactor vendor and that on the other hand its credibility as a fuel supplier was severely damaged by President Carter's retrospective legislation on the supplies of enriched fuel. That leads to a natural partnership between Westinghouse and British Nuclear Fuel Limited (BNFL) whereas in a French dea BNFL would have been in competition with the French fuel company, Cogema.

Nevertheless, Britain's nuclear power capability would certainly have grown

My steam generator's better than yours

AKEY problem affecting the running of pressurized water reactors is corrosion of the steam generators — the massive heat exchangers that transfer heat from the primary coolant to boiler water which, as steam, will then be used to drive turbines.

Any boiling kettle makes a lot of noise and bubbling vibration, and the same is true of steam generators. So the tubes which carry primary coolant through the steam generator must be clamped firmly to stop them vibrating and working loose. Tight clamping leads to corrosion at the clamps. Loose clamping, as originally adopted by Framatome, leads to tubes working loose. (Fessenheim 1, the first French PWR, suffers from this.)

So steam generator design is something of a black art, and the engineers at Framatome think they've mastered it and that Westinghouse has not. Britain, for its PWR design, has adopted the Westinghouse "F-type" steam generator. Framatome analysed the basic design of the model F in 1977-8 and was not impressed. The F-type is still just a design—it has never operated anywhere—and the previous model on which it was based had major problems. Westinghouse has solved some of the problems, but nothing like all of them, according to Framatome engineers. "We believe in rugged systems" said one. "The Westinghouse model is more of a watchmaker's approach, with smaller tubing of which there is no experience."

Westinghouse, however, is unperturbed. The company believes Framatome is wrong on water chemistry, and that the French steam generators will corrode within four years. However, EDF, the operator, is proud of the chemical control procedures. "We are keeping our fingers crossed" say Framatome's engineers.

much faster with Framatome, and now France can gloat over the difficulties facing the British nuclear industry - particularly over the design and cost of safety systems. "We are very sorry for the British" said Coudray. "You can increase the cost of a plant by a factor of two if you are not careful with what you do about safety". According to M. Rémy Carle, a director at EDF, the cost of a Framatome 1,300 MW PWR station is now about FF 5,000 per kilowatt (that is, about £600 million in total) - a price that does not include decommissioning, and assumes a reactor on the coast, but includes all other costs. By contrast, at the recent announcement of the completion of the work of the British PWR task force, whose objective was essentially to reduce the cost of safety systems on the PWR, the figures being quoted were more like £1,000 million.

According to Coudray, the problem is that the British have adopted a four-loop, independently pumped emergency core cooling system (for use in the event of the most serious possible emergency, a "loss of coolant accident" or LOCA). Such a system is easier to explain, and "nicer



Coudray of Framatome — unlike the British, not troubled by a suspicious public

intellectually" that the French safety system, says Coudray. The British system takes five minutes to explain whereas the French takes 3-4 hours — but the French system will do everything the British one will do a much lower cost. Britain has probably adopted the "transparent" system for political reasons, thinks Coudray, to satisfy a suspicious public.

How then has France got so far ahead with its nuclear programme? The research programme undertaken by Framatome in the early 1970s, and which was in full swing by 1975, was crucial to success. A licence brought information; but understanding requires research, said Coudray. Research enabled Framatome to move away from and improve on the Westinghouse design. The exact scale of the French research programme has not been revealed, but it matches anything that Westinghouse has done in the United States.

The company does its research under four framework agreements which allow very rapid changes of direction and commitments of money without the need for constant negotiation and renegotiation. Framatome has its own unique programme

All they're cracked up to be?

You couldn't really have a better recommendation. "Now's the time to buy from the French" said Dr Walter Marshall, chairman of the United Kingdom Atomic Energy Authority, recently, when describing the French design for a pressurized water reactor (PWR). Dr Marshall heads the PWR task force which recently tidied up the design for a British PWR, built under licence from the American company Westinghouse. However, the recommendation is somewhat backhanded.

"The French have been caught with their pants down", said Marshall, referring to a series of cracks discovered in the reactor pressure vessel and steam generators of a number of plants three or four years ago. The result is that the manufacturers, Framatome, are now taking every possible precaution to avoid such potentially dangerous flaws, even though their own "pessimistic" calculations had shown the existing cracks to be unimportant.

Marshall says he has every confidence in Framatome's calculations and experiments, and believes that it has done a "very thorough job". French safety authorities will impose a requirement to inspect existing cracks every time a reactor is shut down for servicing (perhaps every 2–3 years), and Marshall—who made a deep study of the problems of cracking in reactor pressure vessels—believes this will be quite sufficient to prevent accidents.

This is despite the fact that some of the cracks, which appear in the black steel under the stainless steel cladding of the primary containment system, are difficult to observe. They are the ones in the shoulders of the inlet nozzles of the pressure vessel, where cool primary circuit water enters to extract heat from the core: it is simply physically difficult to reach them with the usual ultrasonic and eddy current detectors, unless the whole of the core is removed first. But Marshall believes it would be adequate to monitor frequently only the cracks in the hot outlet nozzles, which are accessible, as a kind of statistical sample of crack growth, while inspecting the inlet nozzles less frequently — say every ten years — a policy which Framatome and EDF will probably follow.

Around 25 of Framatome's earliest reactors are affected by the underclad cracking problem — an example of how design continuity can lead to problems if the design or manufacturing process has a hidden fault. But the cracks may prove to be a very minor hitch compared with another problem which became apparent only this January — faults in the "broches".

These are spring clips which grip and support the fuel cans at the top of the core, and some three years ago in Japan it was discovered that they can be subject to embrittlement caused, perhaps, by neutron bombardment. Until January there had been no evidence of this in Framatome reactors, but then a loose part of a broche was found circulating in the primary coolant in one of the Gravelines reactors. Now the loading of the latest Framatome reactor, at Chinon, has been delayed according to some reports to test and inspect or even to change all the broches before the reactor is started.

Framatome staff do not understand yet what caused the Gravelines broche to break, and whether it was a unique or general fault. But if it was the latter, 37 reactors would be affected, so a crash programme on broche design and testing is now under way. The problem is compounded by the fact that the broches on working reactors will be highly irradiated and so very awkward to replace; but if faulty, they will have to be replaced, for broken broches could jam and interfere with the movement of control rods.

at Creusot-Loire, mainly on materials; a "very privileged and active" programme with the CEA (which is, incidentally, a 30 per cent shareholder in Framatome); a quadripartite agreement, under which onethird of the work is done in the United States and two-thirds in France; and a tripartite agreement among the French partners. Corrosion problems in the primary circuit and steam generators, among the main causes of the low availability of the Westinghouse reactors, were studied under the quadripartite agreement; but surprisingly Westinghouse and Framatome then diverged over steam generator design (see page 300).

The principal reason Framatome has been so successful, though, lies in the extra-

ordinarily tight organization of the nuclear industry in France — a legacy of the work of the previous industry minister, André Giraud. After the battle between CEA and EDF over whether the French gas-cooled or American pressurized water reactor should be chosen - a battle in which de Gaulle championed the CEA, which he had himself set up in 1945, and its gas-cooled system, and which was only won by EDF after de Gaulle resigned - Giraud picked up the pieces of the industry and dried the CEA's tears, giving it total control of the fuel cycle and the development of the fast breeder. He thus created the stable, threelegged organization - CEA, Framatome, EDF - which has put French nuclear power in a world-commanding position.

Superphénix — great white hope

THE world's first commercial-scale fastbreeder reactor, Superphénix, should be generating 1,300 MW of electrical power for the French national grid by 1984. But is it, or will its successors be, economic? And will EDF order Superphénix II, which is already being designed?

Superphénix is in fact owned by an international consortium, NERSA, which is 51 per cent French with the rest largely divided between Germany and Italy (which has the larger share). NERSA is the customer of Novatome, a kind of fast-breeder companion to Framatome (which constructs the PWR). Framatome in fact now controls Novatome, which is also held 34 per cent by the CEA.

Superphénix is expensive: estimates vary, but the price is usually put at around twice that of the equivalent PWR. Of course, it is a prototype; it is alone on its site (PWRs are usually built in fours); and PWR construction is well-developed (Framatome is on the fortieth of its series). But there are some fundamental reasons why Superphénix should be more expensive: it has an extra cooling circuit the second sodium circuit; safety systems are more complex, having to deal with the possibility of sodium fires as well as almost instantaneous nuclear shutdown in the case of coolant loss; and the reactor vessel is stainless rather than ordinary black steel.

Superphénix nears completion

In the long run, the fuel cycle costs should be lower because the fast breeder can generate its own plutonium by neutron bombardment of waste uranium from 'enrichment plants and depleted gasgraphite fuel.

This would also give France a degree of security in uranium supply. French-controlled uranium production is expected to peak at around 4,500 tonnes a year by the mid-eighties, but the PWR programme is expected to need around 8,500 tonnes a year by 1990 and 12,300 tonnes a year by the year 2025 (according to CEA figures). So France is in danger of becoming almost as dependent on foreign uranium supplies

as it is at present on foreign supplies of oil. The introduction of fast breeders, the CEA argues, could reduce this demand to as low as 8,000 tonnes a year in 2025 — provided a fast breeder programme were started soon.

There are two arguments for the fast breeder, says the CEA: that at a certain uranium price the fast breeder will become cheaper per kilowatt hour than a PWR, because of the former's breeding capacity; and that it represents an insurance against loss of uranium supplies. "And you expect to have to pay an insurance premium if you want insurance," says Michel Rapin, CEA director for nuclear applications.

The question of what balance should be struck between these two arguments is ultimately a matter for government and the electricity supplier, EDF, but neither shows any desire for haste although, says the CEA, France needs "a significant programme" of fast breeders by the end of the 1980s to make any impact on French uranium needs by the second decade of the next century.

Moreover, the reprocessing and other pieces of fuel-cycle plant necessary for a fast breeder programme are uneconomic unless built very large. So the CEA is looking for two options: first, a French programme of seven or eight fast breeders, the first of which should be ordered in 1986–87 or, perhaps more practical, an international collaboration on fast breeder construction on about the same scale.

Of course it would appeal to France if the international reactor were close to Superphénix II, and the CEA has already been looking for partners. In Britain, UKAEA officials also believe in international collaboration on fast breeder programmes, and are contemplating agreements with either France or the United States. However, France was reported two years ago to be asking a price of £20–25 million for access to Superphénix technology, a price which Britain at the time rejected.

The reprocessing technology, whose efficiency in extracting plutonium is critical to fast breeder operation, is not seen to be much of a problem at the CEA. Already 15 tonnes of fast breeder fuel have been reprocessed through small fast breeder plants at La Hague (now closed) and Marcoule, and 380 of Superphénix's 400 or so fuel assemblies have been made of plutonium extracted through reprocessing. "We have closed the fuel cycle" says Rapin.

Moreover the breeding efficiency was "sufficiently positive". In recent years CEA scientists - 1,000 of whom work on reprocessing technology - have reduced plutonium losses during reprocessing by a factor of five. "But that is not exactly the problem" says Rapin. To get a lot of plutonium bred by a fast breeder, a thick depleted uranium blanket is necessary. But the thicker the blanket, the less the concentration of plutonium produced in the outer layers of the blanket. And a low concentration of plutonium means a high reprocessing cost. So the best balance has to be struck between the reactor breeding ratio and these costs. Costs, yet again, come back to the centre of the fast breeder stage, and there are certainly some in France who look at Superphénix and think of another costly marvel - Concorde.

Nuclear power — how committed?

WHEN François Mitterrand was elected president of France on 10 May 1981, the nuclear establishment was worried. His socialist party, a relatively new and unknown force in French politics, had committed itself to a fairly strong anti-nuclear policy. Paul Quilès, the architect of the socialists' plan, was effectively seeking a halt to the construction of new nuclear power plants. If this plan had been implemented, the monopoly electricity supplier (Electricité de France, EDF) would have had only 39 GW of nuclear electricity available in 1990, compared with the 59 GW planned by the previous government, and EDF's supplier of nuclear steam supply systems — Framatome — would have found itself with (to say the least) an embarrassing overcapacity. With the export market also sluggish, this would probably have meant the collapse of the French nuclear industry.

However, six days after the presidential election, a strange thing happened. Giscard d'Estaing's centre-right government was in its last few days of limbo, before the elections to the National Assembly (parlia-

ment) and the establishment of the new government. Usually, no major decisions or commitments are taken at such times. But Raymond Barre, Giscard's Prime Minister, unexpectedly announced that the reprocessing facility for spent nuclear fuel at Cap de la Hague, near Cherbourg, was to be massively extended - at an estimated cost of FF 20,000 million (£2,000 million) to cope with 1,600 tonnes of fuel a year. Some of this capacity was to honour contracts already signed with Belgium, Germany and Japan; but the rest implied a strong national nuclear programme. The decision could hardly have been taken without the tacit agreement of Mitterrand, who would have to carry the policy through. So what exactly was the new government's position on nuclear power?

Initially, it seemed as if the Quilès policy might be adhered to. One of the Mitterrand government's first acts was to "freeze" construction on five nuclear sites — Chooz, in the Ardennes near the Belgian border, Cattenom, Civaux, Golfech near the Pyrénées and Le Pellerin. Plans for a nuclear plant at Plogoff in Brittany were

shelved, at least temporarily.

But then other notes were sounded. Jean-Pierre Chevènement, the minister of state for science and technology and a powerful figure in the new government, declared himself solidly behind the French nuclear programme, and all but described the small French environmental movement as "anti-scientific".

Discarding the chaff

Speaking cynically, it is as if Mitterrand had used the environmentalist tendency in his socialist party only so far as was necessary to help build the party and gain power. Now it was possible to let such chaff blow away. The environmentalists do feel themselves hard done by. Giscard had not even lent them an ear. Mitterrand had appeared to listen, and then ignored them. Recently there was a rocket attack on Superphénix, the world's first commercialscale fast breeder power station. Nobody has claimed responsibility, but there are fears at EDF that this means that an extreme section of the environmental movement has now gone underground.

Although the Mitterrand government can in no sense be called "green", it is showing itself to be sensitive to arguments for more democratic control of technical choice. Mitterrand is emerging as a social democrat, although he would not dare to use this label within his party.

In the energy sphere, the evidence for the socialist government's liberalism came first with an energy debate in the National Assembly last October. In contrast to the Quilès policy, the energy policy revealed by Prime Minister Pierre Mauroy and the junior minister for energy, Edmond Hervé, was very mild. It was forced through against the Quilès faction on a vote of confidence. Instead of 59 GW nuclear in 1990 there would be 56 GW. Instead of nine new reactor starts in 1982-83, there would be six. The La Hague development would go ahead. The fast breeder was not debated. The frozen reactor construction sites would be reopened, if a local vote approved; if it did not, it would be up to the regional council (a higher elected authority) to decide; if the regional council did not approve, the matter would come before the National Assembly mechanism designed, it seemed, to get a 'yes'' vote at some level or other.

The environmentalist lobby looked on the black side — this was just the old centralist technocracy by another name. But there is another view, which as time passes comes more and more to the fore. The Mitterrand government is fiercely realistic — while at the same time wanting to make historic and lasting changes in the nature of French society. It is not going to compromise either on economic development or on a slow, emphatic shift of power away from the Paris-based élite, towards the regions, towards the small enterprises rather than the giant conglomerates.

Realism leads the government to under-

Media plug in here

JUST to the side of the Electricité de France (EDF) edifice in Paris is an unobtrusive set of doors. In the case of a major nuclear accident at one of the EDF power stations it is through this door that most of the world's (official) information about it will come.

For behind this door is a FF 4-million (£400,000) communications suite, the brain-child and pride of EDF's chief public relations officer, Mme Marie-Claude Vigna. Mme Vigna began to plan the suite after a French power black-out in 1979 had led to a great press of journalists in a tiny room in EDF headquarters, all attempting to use five telephones at once. If it was like that for a mere power breakdown, what about a "Three Mile Island", Mme Vigna reasoned?

The consequence is that EDF now has probably the most sophisticated "press office" in France. At street level, television vans can simply plug in to a series of channels giving television output from the suite above. Within the suite, sound-insulated rooms take tapes direct from the major wire services such as

Agence France Presse and Reuters, so that journalists — and EDF staff — can monitor media output by the minute. There are of course more than five telephones; a television studio, executive briefing room; and literally every known form of video recording and playback equipment. And officials being interviewed can be relayed information over private monitor screens which are within their vision but not that of the journalists interviewing them (see photograph below).

Outside Paris, hundreds of EDF officials throughout France will be kept in touch by a private videotext signal, sent through the French ANTIOPE system.

A French prefect (regional head man) who was responsible for the region around the Dampierre nuclear power station recently sent out a notice to his constituents telling them what to do in case of a nuclear accident. Don't panic, he said. Keep your children indoors. Don't eat apples from the garden. And, mysteriously, don't telephone. Well, there'd be no need to with what EDF has in Paris, would there!

Energy minister Edmond Hervé confronts the press in EDF's media room



stand that a country 70 per cent dependent on foreign fuel, whose healthy non-oil trade balance is wrecked by the cost of oil imports (99 per cent of oil is imported), must attempt to internalize the costs of energy production. Nuclear electricity is part of the solution. The government is also realistic enough to know that the wholesale destruction of the nuclear industry would be immensely demoralizing to French people - who, while being as suspicious of nuclear power as the next nation, see its success as one of the great symbols of French strength. France has been overrun by a foreign power three times since the Revolution, and no Frenchman is going to let it happen again - either militarily or economically.

Handling the public

On the other side of the equation, the small group of men at the head of EDF, the CEA and Framatome, who effectively control the nuclear development of France, are gradually being forced to pay serious attention to the general concerns of the public and the particular demands of the regions

where nuclear facilities are being sited.

For example, when the government says it will "reinforce the independence" of the Conseil Supérieure de la Surété Nucléaire (the senior nuclear safety council) and "modify its composition", it means that it really will attempt to detach the safety council from its tutelage to the nuclear establishment. The proposal to create a "safety director" under the control of EDF may be looked at a little askance, as may guarantees of the independence of the Institut de Protection et de Surété Nucleaire, the technical safety assessment body which exists under and has constant exchange of staff with the Commissariat à l'Energie Atomique; but the establishment of local information commissions on each nuclear site could be taken more seriously.

These commissions are proving slow to set up (so far there is one at La Hague and another at Nogent-sur-Seine) but, according to commitments made by the government in the energy debate, they will be pluralist, "contradictoire" (in other words allowing serious debate and close questioning), independent and permanent.

Hard bargaining at Golfech

The apparently anodyne decision to subject the five frozen construction sites to a hierarchy of democratic assessments, from local to national level, until at some stage somebody said "yes", has also proved a thorn in the EDF flesh. The votes went in favour of continuing construction at Chooz, Cattenom and Civaux. But at Golfech, near the border with Spain, and Le Pellerin, there were marginal local votes against. So the question went to a higher level — the regional council. The Le Pellerin council voted for the reactor. But Golfech, while eventually agreeing, drew blood from EDF in the process.

The "blood" took the form of a contract with EDF — the first ever such binding arrangement between EDF and a region — which guarantees the locality 40 per cent of the jobs that will eventually be created at



Michel Rapin of CEA says the nuclear programme is stronger under Mitterrand

the power station, a large fraction of the construction work and EDF support for amenity development. EDF is currently seeking sites for five reactors at 1,300 MV and one at 900 MW — the programme agreed at the National Assembly for 1982–83 — and the Golfech deal may prove to be an awkward model. EDF, however, argues that it has always tried to place jobs and contracts around the region of a nuclear site. The real difficulty, says EDF, will be to find 40 per cent of its nuclear engineers in the Midi-Pyrénées.

The latest twist in the tail is literally there—at the tail end of the nuclear fuel cycle. Waste disposal has been neglected in France (although the Marcoule process for the vitrification of highly active waste has been adopted by Britain). Edmond Hervé, minister for energy, has asked the CEA to provide, within a few weeks, an outline plan for the management of nuclear wastes. No such plan exists at present, it seems. Within 18 months, two or three disposal sites must be selected, promising a new political problem for nuclear power.

Some in the nuclear establishment, however, are quite sanguine about the developments. Michel Rapin, director for nuclear applications at the commissariat, believes the new political approach will actually strengthen the nuclear programme, by providing a degree of democratic assessment. Once there has been a vote, who can disagree? All very well, while the votes are "yes"...

Trebling renewable energy by 1990

ONE of the most surprising but rational moves made by the Mitterrand government in the energy field has been its appointment of Michel Rolant, a union activist in his late 40s, as the head of a completely revamped organization to promote renewable energies in France.

Rolant was an agricultural worker who took a strong interest in labour relations, and early on became general secretary of the federation of agricultural workers, a branch of the liberal union, CFDT. But his attention shifted to industry and employment, and he established a reputation, among the pro-nuclear lobby, of being totally anti-nuclear. Certainly, as CFDT's number two, Rolant moulded the union to form effectively the only organized opposition to the nuclear establishment — a position which it will retain despite Rolant's departure.

Electricité de France is turning a rather jaundiced eye on Rolant's appointment — the cosy club of grandes écoles men at the top of the ministries and the great industries is being jostled these days by one or two workers with some peculiar ideas — but for the government the move is really a master-stroke (and, by the way, one long advocated by CFDT). For, from the nuclear point of view, one of the principal irritants has been mollified: he

to be determined. The CFDT recommends a balanced regional and central organization, with 22 regional branches each with perhaps 50–100 staff, and a 600–700 strong national body. (By comparison, COMES and AEE presently have about 500 staff mostly in Paris). The new body should also be capable of doing research on its own behalf, together with the major research organizations.

Moreover if capital loans could be arranged on the kind of terms on which they are offered to Third World nuclear purchasers — terms such as 8 per cent interest to begin five years after completion of a reactor as were offered to Korea, for example — then wonders could be worked, say the enthusiasts.

In fact COMES announced an objective at the end of last year: to treble the funds devoted to solar energy between 1981 and 1985. The most promising areas for development were the production of petrol and methane from biomass, particularly wood waste of which France has a particularly good supply. But Rolant will want to see all alternatives, including energy saving, in perspective, and perhaps COMES's priorities will not be his. The government is certainly committed in principle to a trebling of funds for renewable energy projects, although the allocation of the

Consumpti	ion of prin	ary energy:	evolution an	d objectives	*
	1970	1974	1980	1981	Plan 1990
Coal	38.1	31.6	34.0	33.5	35-40
Petrol	87.5	113.2	102.1	93.0	70-75
Gas	9.3	16.0	23.6	24.6	31-40
Hydroelectrics	12.4	12.5	16.0	15.0	14-15
New energies	2.0	2.1	3.2	3.4	10-14
Nuclear	1.2	3.1	12.9	19.5	60-66
Total	150.5	178.5	191.8	189	232
Proportion from indigenous sources (%)	65.6	75.0	71.0	68.0	45-50

^{*}Values are in million tonnes of oil equivalent

is now part of the government club and must be expected to obey the rules. And from the alternative energy point of view, here is a man who is a passionate and serious advocate of such forms of energy who must now turn his dreams into reality. If they work, well, that means more energy for France.

In fact the government's energy plans for the next decade are extraordinarily ambitious both in respect of their nuclear component and their renewables (see table). The energy supply from renewables — such as solar power and biomass — is expected at least to treble by 1990 compared with 1981, and M. Rolant will be presiding over that growth. Rolant will have available to him COMES, the commission for solar energy, and the AEE, the agency for energy saving, which will be formed into a new organization whose exact definition and scale have yet

cash will not be known until mid-1982.

No doubt Rolant will have considerable influence on this, and perhaps his agricultural background will influence him (his colleagues at CFDT deny it); but it has been clear for some time that in renewables France is concentrating on the conversion of biomass. Of the 10-14 million tonnes of oil equivalent expected from renewables in 1990, half will come directly from the use of wood, and a further 2 million tonnes of oil equivalent from wastes. Surprisingly, however, of FF 35.2 million (£3.5million) research ministry funds available for renewables research in 1981, only FF 0.2 million (£20,000) went to support biomass research. The largest sum (around half) went to coal. COMES spent FF 50 million (£5 million) on biomass development in 1981, but only a small fraction of that went on basic research. This may change.

NEWS AND VIEWS

Demon nuclei— spurious excitement?

GAMERA A COMPLEX

BE A P.C. No. Col.

CIR RAPY COMPLEX

Description of t:

Chambe a compression of the complex
from Frank Close

CAN quarks, the hypothetical ultimate constituents of all nuclear matter, cluster together and form 'demon' nuclei - an exotic form of nuclear matter in which individual protons and neutrons cannot be discerned? The existence of such matter has been recognized as a possibility for some time, but now great excitement has been caused by a claim that 'demon' nuclei may actually have been detected. Early this year S. Fredriksson and M. Jandl of the Royal Institute of Technology in Stockholm (Phys. Rev. Lett. 48, 14; 1982) predicted, from a new theoretical approach, that demon deuterons might have been responsible for anomalies seen both in cosmic experiments and in recent experiments at the Lawrence Berkley Laboratory. However, an even more recent paper suggests that the excitement may be premature. H. J. Lipkin of the Weizmann Institute (WIS Report 82/2, January; 1982) raises powerful theoretical objections to the existence of demon deuterons and shows that whatever is responsible for the experimental anomalies, it cannot be demon nuclei.

The ways in which quarks cluster together to build up nuclei can be thought of as similar to the ways that electric charges cluster together to build atoms and molecules. In the latter case, electromagnetic attraction between opposite charges causes electrons to encircle the atomic nucleus to build up atoms whose total electrical charge vanishes. Neighbouring atoms are held together by three varieties of forces, each of which has its origin in the fundamental electromagnetic force. Neighbouring atoms with an excess or deficiency of an electron can be directly attracted (ionic force); electrons can be shared among atoms (covalent force); or there can be an asymmetric distribution of charge within the overall neutral pattern (van der Waals' forces).

Quarks carry a property called colour which is similar to electrical charge except that instead of there being just one variety of positive charge there are three varieties (red, yellow and blue) of positive or negative colour. Unlike colours attract just as unlike charges do. Quarks carry positive

colours and their antimatter equivalent, anti-quarks, carry the negative or complimentary 'anticolours'. Quarks and antiquarks will thus be mutually attracted and form the variety of matter known as mesons, of which the pion is the most familiar example. However, because of the threefold nature of the colour charge there are possibilities for quark clusterings which do not have a direct analogue in electromagnetism.

For example, two 'red' quarks will repel one another, but if a red and yellow one are nearby they will be mutually attracted. If a third quark carrying a blue colour is near to this pair, then the red and yellow, yellow and blue, and red and blue will all mutually attract one another and can form a stable system known as a baryon, of which the neutron and proton are the lightest examples. If a fourth quark comes near to this system it will be repelled by the red-, yellow- or blue-coloured quarks depending on which colour it is itself carrying. Three quark clusters are thus very stable and this is the source of the baryon's existence.

In the cluster of red, yellow and blue quarks that forms the baryon, the total colour has cancelled out leaving no net colour: just as the electrically neutral atom results from electromagnetic forces. In turn, the forces that cluster adjacent neutrons and protons to one another to form nuclei are the colour analogues of the covalent or van der Waals' forces of electromagnetism. An important difference between colour forces and electromagnetic forces is that colour ions appear to be forbidden to exist as free entities in nature. Thus individual coloured quarks or pairs of quarks which carry net colour have no free existence, although this need not prevent the existence of colour ions within densely packed clusters - so long as the total colour of the whole cluster vanishes. Indeed, within the proton there exist two quarks with net colour which are attracted to the third quark which itself

carries colour; in this sense the proton is built of a di-quark coloured ion and a quark coloured ion. Could there, then, be ionic forces between di-quarks within nuclear matter? The simplest example would be in a cluster of six quarks of which the most familiar example is the deuteron; three quarks form an uncoloured cluster known as the proton and the remaining three form an uncoloured cluster, the neutron. The neutron and proton then attract by the residual colour forces, analogous to covalent forces (pion exchange) or to van der Waals' forces (about which there is much theoretical discussion but not total consensus vet). However, there is also the possibility that this total of six quarks, instead of forming two uncoloured clusters of three, might form three colour ions of two! Thus the deuteron, which is two sets of 'tri-quarks', could have a demon brother which is a set of three di-quarks. The di-quarks carry colour and will be strongly attracted to one another, in a manner analogous to ionic forces. They will be bound very tightly in a cluster of six quarks, in contrast to clustering into groups of three where the residual force would be expected to be weaker. Thus, it seems in principle possible that a stable cluster of three di-quarks exists in nature, perhaps lighter and more stable than the familiar deuteron.

This idea is at the root of the paper by Fredriksson and Jandl. The authors predict that their demon deuteron will be light enough to be almost stable and might be responsible for the anomalous nuclear interactions that have been seen in cosmic experiments (the Judeck effect) and in recent experiments at the Lawrence Berkley Laboratory (E.M. Friedlander et al. Phys. Rev. Lett. 45, 1084; 1980). When a high-energy nucleus collides with an emulsion target, it was discovered that about six per cent of the fragments created in the collisions travelled only very short distances, much less than normal, suggesting that they were somehow anomalous with very high reaction cross-sections, long lifetimes and stable against strong decays. Fredriksson and Jandl suggest that the large interaction cross-section arises

Frank Close is Principal Scientific Officer in the Rutherford Appleton Laboratory, Didcot, Oxfordshire OX11 0QX. because 'demon nuclei' are produced.

Even more recently, important difficulties with this suggestion have been raised by H.J. Lipkin of the Weizmann Institute. Crucial in creating a demon deuteron from three di-quarks is the role of the Pauli principle. Having all three di-quarks in s orbitals violates the Pauli principle and so the authors suggest each di-quark is in a p orbital, the three of them coupling to a total L = 0 state with negative parity.

Lipkin points out that as there are only two independent orbital angular moments in a three-body system, then the $J^p = 0$ -object made from three $J^p = 0$ di-quarks cannot exist. The so-called demon state is a spurious state of centre-of-mass motion in which the centre-of-mass of the three diquarks is oscillating in a p-wave. When this

spurious excitation is removed by putting the centre-of-mass in an S-state then one obtains an allowed state with total angular momentum of one. This isoscalar $J^p=1^+$ has the same quantum numbers as the deuteron, might very well be mixed into the deuteron's wave function and be detectable in measurements of the deuteron's form factors. However it would not be expected to occur as a separate metastable state.

Thus the observation of anomalous nuclear processes on the one hand and the possible occurrence of new multi-quark configurations in nuclear matter on the other remain, for the moment, unconnected. If demon nuclei do exist it seems that they do not occur in the way that the Swedish group claim.

noted the resemblance of many features of the skull and skeleton of the thecodontian Chasmatosaurus (Proterosuchus) to those of rhynchosaurs. Carroll^{4,5} developed these ideas further and demonstrated close similarities between Heleosaurus, a late Permian eosuchian, and the early Triassic thecodontian Euparkeria. Although clearly similar to Youngina, Heleosaurus had a dentition very like that of Euparkeria and it may have been capable of an upright posture, an advanced feature of many archosaurs. Gow⁶ redescribed Youngina, and also suggested that it was close to the ancestry of archosaurs.

New information on the affinities of rhynchosaurs and sphenodontids is also damaging to the integrity of Romer's Lepidosauria for it is believed that these two groups are particularly closely related. Carroll⁷ redescribed Noteosuchus, a partial skeleton from the early Triassic of South Africa, and interpreted it as the oldest known rhynchosaur. Its ankle structure is similar to that of Chasmatosaurus (Proterosuchus). Carroll stressed that there is no evidence for a close relationship between rhynchosaurs and sphenodontids and that supposed shared characters are either primitive features of all diapsids, or they have been wrongly interpreted. For example, the living Sphenodon has acrodont teeth (fused to the summit of the jaw bone), while rhynchosaurs had deeply rooted teeth. Sphenodon has a row of teeth on two

The Diapsida: revolution in reptile relationships

from Michael J. Benton

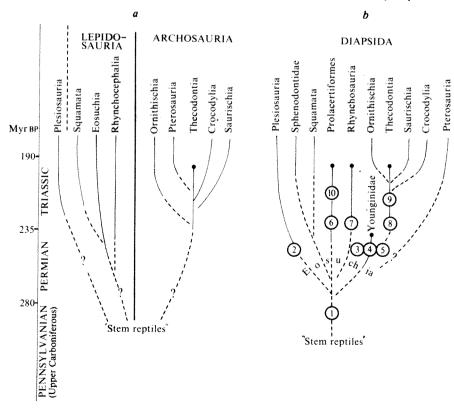
DISCOVERIES of new fossils and reinterpretation of existing data suggest the need for a major change in the traditional view of the relationships and ancestry of the lizards, snakes, crocodiles, dinosaurs and many extinct groups of reptiles. In standard textbooks (for example, Romer's Vertebrate Paleontology), the reptiles are divided into subclasses according to the number of openings behind the eye sockets. Those with two openings, the Diapsida, are further divided into two groups, the Lepidosauria (including lizards, snakes, sphenodontids, rhynchosaurs and eosuchians) and the Archosauria (including the thecodontians, crocodiles, dinosaurs and pterosaurs), that are thought to have diverged from separate ancestors as early as the Upper Carboniferous (Pennsylvanian; see Fig. 1a). The new view (summarized in Fig. 1b) is that all diapsids have a common ancestry and that their classification will have to be completely revised.

Romer¹ considered that lepidosaur groups, including the lizards, snakes, sphenodontids and rhynchosaurs (medium-sized 'beaked' and probably herbivorous reptiles of the Triassic), all derived from the eosuchians, a mixed group of primitive forms, of which Youngina from the late Permian of South Africa is usually regarded as typical. In contrast, archosaur groups, including the crocodiles, dinosaurs and pterosaurs, were considered to be derived from thecodon-

tians, such as the early Triassic Chasmatosaurus (Proterosuchus).

More recent results now suggest that both lepidosaurs and archosaurs had common ancestors among the eosuchians^{2,3}. Cruickshank³ particularly

Fig. 1 Evolution of some major groups of reptiles, according to Romer¹ (a) and to recent work (b). Animals mentioned in the text are shown in b as follows: 1, Petrolacosaurus; 2, Claudiosaurus; 3, Youngina; 4, Thadeosaurus; 5, Heleosaurus; 6, Prolacerta; 7, Noteosuchus; 8, Chasmatosaurus (Proterosuchus); 9, Euparkeria; and 10, Tanystropheus.



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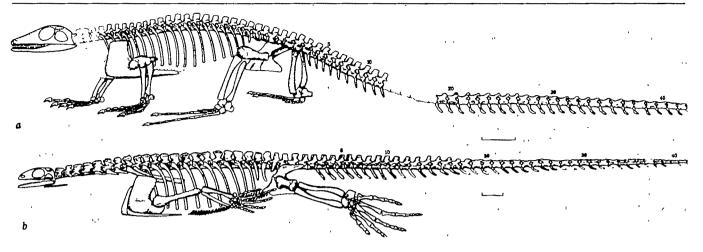


Fig. 2 Restorations of the skeletons of Thadeosaurus (a) and Claudiosaurus (b) in side view. Claudiosaurus is shown in a swimming pose. Scale bars are 2 cm. The similarities between Claudiosaurus, supposedly the earliest plesiosaur, and Thadeosaurus, a contemporary eosuchian from Madagascar, suggest a close relationship. After Carroll¹⁴

separate bones of the palate (the maxilla and the palatine), while the multiple rows of teeth in rhynchosaurs are all on the maxilla. Sphenodon appears to show more affinity with ancestral lizards.

New evidence also suggests that the Pterosauria should be removed from the Archosauria and recognized as a distinct group. As early as the late Triassic, pterosaurs from Italy, described by Wild8, had all the special adaptations of the group for flight and had diversified into two quite different stocks. Wild suggested that pterosaurs originated directly from the eosuchians in the early Triassic since their anatomy suggests that they derived from small running insectivorous forms. The thecodontians of the Triassic were generally too large or otherwise unsuitable as ancestors, and there would probably have been insufficient time for a radiation from them to have occurred.

We can now discern four separate lineages of diapsid reptiles that radiated from the Eosuchia in the late Permian: pterosaurs, thecodontians, rhynchosaurs, and a group made up of lizards, snakes and perhaps sphenodontids. But how do we define the Eosuchia?

Romer¹ included a broad group of Younginiformes, the Prolacertiformes (centred on Prolacerta, an advanced form from the early Triassic of South Africa, that shows some parallel features to lizards) as well as some later aquatic forms. Evans⁹ noted that the Eosuchia have no diagnostic advanced characters and can only be defined by the absence of features typical of other groups. Thus, she broadened the Eosuchia to include all diapsid reptiles except the archosaurs, the lizards and snakes. Further work may enable us to extract some of the motley collection of reptile families from the eosuchian rag-bag when we understand more about the diapsid radiations in the Permian and Triassic.

The confusion over the definition and limits of the Eosuchia makes it difficult to decide whether the Eosuchia had a

common ancestor, but an apparantly suitable form exists. Petrolacosaurus from the late Pennsylvanian (260 million years ago) of Kansas has been redescribed recently on the basis of much new material 10. It was clearly a diapsid, but certain features also indicate its ancestry among the 'stem reptiles'. Chatterjee11 has included Petrolacosaurus in the Prolacertiformes, together with several Permian and Triassic reptiles. He regards the enlarged prolacertiform group as a lineage evolving separately from other eosuchians. Wild12 has independently come to a similar conclusion on the basis of a re-study of the curious long-necked Tanystropheus from the middle Triassic

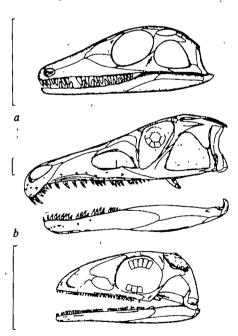


Fig. 3 Side views of the skulls of Youngina (a), Chasmatosaurus (Proterosuchus) (b) and Claudiosaurus (c), an eosuchian, a thecodontian and what may be the earliest plesiosaur respectively. These early representatives of the groups show considerable similarity. Scale bars are 2 cm

After Carroll¹⁴ and Cruickshank³.

period of Switzerland.

One recent study adds a further dimension to the diapsid reptiles by suggesting that the aquatic plesiosaurs, previously of uncertain affinities, derived from the Eosuchia. Carroll¹³ described two reptiles from the late Permian of Madagascar: Thadeosaurus, a younginid eosuchian, and Claudiosaurus, which he interprets as the first plesiosaur (see Figs 2,3). Claudiosaurus resembles younginids in its general anatomy, but it lacks the lower temporal bar and has a closed palate - typical plesiosaur features. Although it does not show all of the particular adaptations for aquatic locomotion that plesiosaurs had (paddle-like limbs, streamlined body, strong tail), certain features suggest that it was a swimmer (poorly ossified wrist and ankle, proportions of the limbs, small skull, long neck). The ancestry of plesiosaurs has been problematical, but Carroll makes a strong case for their derivation directly from diapsid reptiles during the Permian.

There now seems to be little evidence for the separate status of the Lepidosauria and the Archosauria, and it appears that all diapsid reptiles can be derived from Petrolacosaurus. Thus, we may re-instate the Subclass Diapsida, established by Osborn¹⁴ in 1903. This group will now contain a large number of forms, many of them poorly known, and future work must concentrate on sorting out their relationships.

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Two bar mitzvahs for tubulin

from Chandler Fulton

Among the thirteen years since tubulin was named1, the past year has been particularly exciting*. Complete sequences have been reported for both α -tubulin and β -tubulin subunits, the heterodimer of which constitutes tubulin, and it is now clear that cells contain multiple tubulins encoded in multiple genes.

Tubulin is the major building block of microtubules. All eukaryotes have microtubules, so the first eukaryote

*This report is inspired by two meetings which might be said to have celebrated tubulin's reaching, in human terms, the age of responsibility: a joint symposium of the Embryology and Physiology courses at the Marine Biological Laboratory on "Organization and Expression of the Genes for Tubulin", organized by R. Raff and J. Rosenbaum and held at Woods Hole on 17 July 1981, and a European Molecular Biology Organization Workshop, "Microtubules in Microorganisms", organized by P. Cappuccinelli and held at Porto Conte, Sardinia, on 8-12 September 1981.

presumably had tubulin, probably already utilized for the conserved functions of mitosis and flagellar movement. Yet the origin of tubulin is lost in antiquity (about 3×109 BC). Margulis2 has provocatively proposed that microtubular structures arose through integration of an endosymbiotic spirochete into a protoeukaryote, but there is only tenuous evidence to support this view.

Three groups have reported the complete sequencing of tubulin subunits from brain, both by direct analysis of pig brain tubulin subunits3,4 and by sequencing of

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100 years ago

MAXIM'S SELF-ACTING FIRE-**EXTINGUISHERS**

However certain it is that fires in theatres will never be completely suppressed, we may still hope by energetic measures and systematic arrangements to lessen both their number and their danger.

The stage is undoubtedly the most dangerous point, from the very nature of the materials composing it. M. Maxim's proposes to institute such a preventive system that, as soon as a fire begins to show itself at any given point of the stage, the accident will itself produce automatically and instantaneously a series of mechanical movements sufficient to flood the threatened part with water, and arrest the progress of the fire.

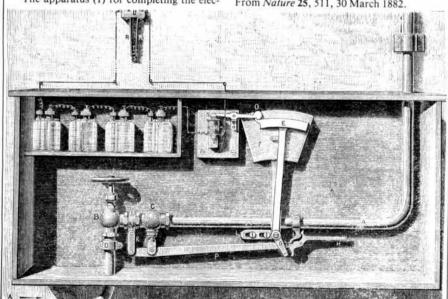
The self-acting *Electric System* he proposes is composed of three distinct parts: (1) an apparatus which completes the electric circuit, under the action of the raising of temperature caused by the fire; (2) a self-acting supplycock, to send water into the system of pipes from the main pipe in the street; (3) an arrangement for opening the discharge-pipes upon any part which is in danger.

The apparatus (1) for completing the elec-

tric circuit is extremely simple. It is composed (Fig. I) of two metallic plates, R. R. forming springs, and separated by a small piece of fusible metal, s, which is isolated from the plates by paper, or any other isolating body. The heat caused by the fire melts the metal, and the plates coming into contact, the electric acts upon the automatic supply-cock. This self-acting supply-cock (Fig. I) is composed of an electro-magnet, M. which, under the action of the current, becomes active, and attracts the arm N, thus setting free the lever, E. The weight, F, then turns from right to left, and, after describing the quarter of a circle, falls upon the lever, H, whilst the part, G, removes a check which has kept the lever, P. in position. Under the action of the weight, F, and by means of the lever, P, which has its fulcrum in the point, J, the supply-cock, c, is turned, and the water rushes into the pipe, A, to be discharged above. The cock at B, which is worked by hand, serves to stop the supply when the fire is extinguished.

The water pouring upwards into the pipes ought to be discharged at the point where the fire appears. Ordinary perforated pipes may be used, and the system of pipes may be disposed around the stage.

From Nature 25, 511, 30 March 1882



cDNA clones to chick brain tubulin mRNA5 and rat brain α-tubulin mRNA6. One immediate consequence is that the molecular weight of both subunits is reduced from the widely quoted 55,000 to 50,000 (450-451 and 445 amino acid residues for α - and β -tubulin respectively). The complete sequences confirm earlier insight that the two subunits share considerable homology3-5. They also support the view that tubulin is among the most conserved of proteins; in 270 million years of separate evolution leading to chickens and pigs, only about 7 of the 855 sequenced residues have been altered. The available information suggests that the subunits diverged from a common. unknown ancestor, and since then their evolution has been held in check, perhaps by functional constraints on the protein. Preliminary evidence based on peptide mapping suggests that protistan tubulins have a-subunits quite different from vertebrate tubulins7. It seems probable that the sequencing of additional tubulin subunits will shed light not only on the structure and function of tubulin but also on the evolution of eukaryotes.

The amino acid sequences and molecular cloning experiments both support the conclusion that many eukaryotes contain and express multiple tubulin genes to produce tubulins that differ slightly in primary structure, an hypothesis that seemed improbable to most when it was proposed six years ago. The protein sequences indicate at least four different α-subunits and two different β-subunits in pig brain tubulin3,4. There are at least two α - and two β -tubulin genes in Chlamydomonas8.9, four of each in Drosophila and chickens, and more than ten in sea urchins, rats and humans10. In Drosophila and in chickens, the multiple tubulin genes are dispersed in the genome (see, for example, ref. 11). All four a-tubulin genes are expressed in Drosophila12, and E.Y. Lai and I have found that at least two β-tubulin genes are expressed during the differentiation of Naegleria amoebae to flagellates. Although the coding regions of tubulin genes are sufficiently conserved for complementarity to be detected among diverse eukaryotes, from yeast to humans5, the flanking regions are divergent even within a single organism (see, for example, ref. 12). The existence of multiple tubulins and tubulin genes requires revision of many of our ideas about tubulin, as well as re-interpretation of old experiments, especially those that measured tubulin pools and synthesis as if tubulin were a homogeneous protein. It also raises questions about the role of multiple tubulins, in particular whether the different tubulins are used selectively in building microtubular organelles. Actin too has been found to be a family of genes and proteins13, which raises similar issues for this protein.

Instructive tubulin mutants are beginning to appear. A testis-specific

β-tubulin mutation has been described in Drosophila¹⁴, which provides evidence that there is more than one functional β -tubulin in this organism. Oakley and Morris¹⁵ have carried out ingenious work on benomylresistant mutants of Aspergillus. A class of these mutants was shown to contain mutations of a β-tubulin structural gene (benA). Some benA mutations alter the isoelectric point of β -tubulin, and these always alter the mobility of two β -tubulin isoforms; thus both isoforms are products of the benA gene. Revertants of a benA mutation were used to isolate a mutation in an α-tubulin gene. A temperature-sensitive benA mutation, with hyperstable microtubules, was used to show that, as Inoué has long argued, microtubule depolymerization is involved in chromosome movement.

The most-studied tubulins have been isolated from vertebrate brains, which are rich in the protein, by cycles of assembly into microtubules and disassembly, but until recently attempts to isolate tubulin from the cytoplasm of protists have been unsuccessful. The systematic isolation of polymerizable tubulin from Physarum amoebae16 and from yeast17 should encourage future attempts and provide opportunities for much-needed comparative studies. Although many properties of tubulin and microtubules, from antigenic determinants to ultrastructure. have been conserved during the evolution of eukaryotes, others seem to vary. For example, whereas colchicine inhibits the assembly of microtubules in diverse eukaryotes, it has little effect on the polymerization of tubulin in many protists. In some of these protists polymerization of tubulin is quite sensitive to benomyl^{16,17}. Although we continue to learn from the exhaustive studies of brain tubulin, novel studies of other tubulins are likely to change our perspective.

The cylindrical walls of microtubules usually contain 13 protofilaments (strings of tubulin), but many exceptions have been reported. Kilmartin found that when

yeast tubulin is polymerized in vitro, in the first assembly there are 12 protofilaments. whereas there are 13 in the second cycle¹⁷. In Caenorhabditis, Chalfie¹⁸ found that although most of the neuronal microtubules have only 11 protofilaments, six touch-receptor cells have microtubules containing 15 protofilaments. Experiments using mutants, colchicine and benomvl indicate that these 15-protofilament microtubules are important for sensory transduction. Proteins associated with microtubules, of which many are known, may determine the number of protofilaments, but we have little understanding of this process. The temporal and spatial orchestration of the intracellular assembly of tubulin into precise arrays of microtubules remains even more mysterious, although elegant descriptive studies of these arrays are beginning to address the issue (for examples, see ref. 19).

In the few years that tubulin has been studied, we have just become acquainted with the possibilities explored during the evolution of cells that utilize this conserved structural protein in cell shape and motility. Much has been learned, but how long will it take us to understand the important features of a crucial eukaryotic protein that has been experimented with for billions of years, among countless billions of organisms?

Was Precambrian seawater different?

from Peter J. Smith

MINERALOGICALLY: dolomite is a simple carbonate of calcium and magnesium. As far as its genesis is concerned, however, it is rather more of a puzzle. Although common in Precambrian rocks, dolomite is apparently no longer being laid down, except in a few marginal environments such as supralittoral zones and ephemeral lakes, even though seawater is supersaturated with it and, on thermodynamic grounds, it should be precipitated in preference to calcite or aragonite (forms of calcium carbonate). Moreover, when it has formed in recent (that is, Phanerozoic) times, it has usually been of the replacement type, which means that it began as calcite or aragonite and later had part of its calcium replaced by magnesium. The geological consensus is that the amount of primary dolomite precipitated directly from seawater during the Phanerozoic has been insignificant compared with the quantity of replacement dolomite generated; and observation shows that, over the same time span, the dominant primary precipitate from seawater has been calcium carbonate (limestone).

This modern preference for limestone has led many, if not most, sedimentologists to suppose that the same bias has always held. The preponderance of dolomite rock (dolostone) over limestone during the Precambrian is then readily explained in replacement terms; limestone has always been the preferential primary product but, being older, the Precambrian representatives have had longer to make contact with magnesium-rich, dolomitizing fluids. There is, however, at least one other possibility — that dolomite was itself the principal carbonate precipitate during the Precambrian because Precambrian

Phanerozoic successor. In that case, Precambrian dolomites (chiefly primary) and Phanerozoic dolomites (chiefly replacement) should be different in terms of their textures, fabrics and isotopic ratios.

That they can indeed be different has now been demonstrated by Tucker

seawater was chemically different from its

now been demonstrated by Tucker (Geology 10, 7; 1982). The Beck Spring Dolomite, which now outcrops in eastern California, is thought to have been laid down 1,200-900 million years ago in various environments ranging from subtidal shoals through lagoons to tidal flats. To Tucker, however, its most conspicuous feature, which it shares with many other Precambrian dolostones, is the extent to which its sedimentary structures have managed to preserve their detail on both broad and microscopic scales. There is no clear evidence of the destruction of original texture and fabric, nor is there any sign of subsequently imposed replacement fabric. Indeed, if other types of analysis had not shown the carbonate to be dolomite of Precambrian age, the Beck Spring Dolomite could well have been taken for an undolomitized Phanerozoic limestone. This is not to say that there have been no changes at all, for there is some evidence of neomorphism. But neomorphism does not necessarily mean mineral change; it could simply have involved wet recrystallization of dolomite.

On the basis of petrographical examination, then, the simplest interpretation is that the dolomite is primary and not a calcium carbonate replacement — a view that receives more than adequate support from isotopic data. Using a scalpel and a dentist's drill, Tucker went on to extract from the Beck Spring Dolomite samples of the various components (micrite, pisolites, fibrous dolomite, internal sediment and so on), which were

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then subjected to carbon and oxygen isotope ratio measurements using standard procedures. The results were, of course, numerical in detail, but the general picture is easily sketched. The data revealed distinct isotopic trends with all examples of each component falling within a distinct and limited field.

That such systematic information can be obtained at all emphasizes the importance of taking an inhomogeneous rock apart and analysing the components separately. Failure to do this in the past has often produced fuzzy data with large variations liable to misinterpretation. That such systematic information was actually obtained in this case, however, indicates that no major re-equilibration has occurred since the dolomite was laid down during the Precambrian. Since that time there has been no extensive interaction with meteoric waters, no deep burial, no more than minimal metamorphism and little deformation.

All the indications are, therefore, that the isotopic ratios and trends are original. What's more, those ratios and trends bear a striking similarity to those from primary Phanerozoic limestones. Tucker thus concludes that the Beck Spring Dolomite and, by implication, the many other Precambrian dolomite rocks with comparable properties were precipitated directly as dolomites and thus not generated by the dolomitization of directly precipitated limestones. And as the most important control on carbonates is the isotopic composition of the water from which they are precipitated, the inevitable, or at least the simplest, overall conclusion is that the Precambrian seawater that favoured the production of Ca-Mg carbonates (dolomites) must have been chemically different from the Phanerozoic seawater that gave, and gives, rise to Ca carbonates (limestones).

Unfortunately, Tucker does not elaborate on the difference, except to note the general consensus that the seawater characteristics favouring dolomite precipitation would include a higher than usual Mg/Ca ratio, a higher CO₂ partial pressure, a higher temperature and a lower SO₄²- content. So mysteries remain. Why does dolomite not precipitate from modern seawater if, as Tucker claims, it is favoured thermodynamically? Why experiments to synthesize dolomite under simulated sedimentary conditions merely resulted in an imperfectly ordered, Ca-rich version of dolomite ('proto-dolomite')? Why, if dolomite was the preferred precipitate during the Precambrian, were limestones nevertheless sometimes generated? And when (and why) precisely did the preference change from dolomite to limestone?

What the answers to these questions might say about dolomite is of comparatively minor importance, but what they might say about the evolution of seawater composition could be a rather bigger issue.

Intermediate valence compounds as applicable materials?

from Michael Kelly

UNDER modest pressures, the normally semiconducting samarium sulphide undergoes a first-order phase transition to a metallic state. Thus by rubbing or scribing the surface of this material, an intrinsic metal-semiconductor contact can be generated and can subsequently be removed by temperature excursions of approximately 100°C.

Possible applications of this and other surprising properties of the so-called intermediate valence compounds, especially those involving compounds and alloys of the rare-earth elements, were outlined by P. Wachter (Zurich) in the closing session of a one-day meeting* devoted to the physical properties of the these materials. As the name implies, the ionic constituents of intermediate valence compounds may carry non-integral charges. Briefly, in the solid environment, only a small energy difference may be involved in the transfer of an electron from an incomplete f shell of a rare-earth atom to the available states of the valence band. The result is that there may be dramatic anomalies of physical properties not so far explained by conventional valence band theory.

Wachter opened the meeting with a review of recent work by his team on semiconducting intermediate valence compounds. Because of the weak interactions of electrons in the f shells of adjacent rare-earth atoms, the characteristic energy gaps are of order 10 meV, corresponding to temperatures of order 100K. These are similar to energies associated with magnetic ordering and farinfrared optical absorption, so that small temperature changes can induce substantial changes in many physical properties. The metal-insulator transition under pressure is accompanied by a volume change of order 10 per cent, because of the different size of the rare-earth atom in its different charge states. As an incipient instability, it causes the elastic properties to be anomalous under ambient conditions. Compressibilities are enhanced, some elastic constants are negative and finite wavelength vibrational instabilities are encountered. G. Saunders (University of Bath) reported on these anomalies as the transition is approached. Possible applications as pressure sensors and phonon generators can be envisaged.

The rare-earth metals and their alloys (including those exemplified by Eu Cu₂Si₂) have optical, magnetic and electronic

transport properties that vary as the valence changes (reviewed by D. Wohlleben, University of Koln, and F. de Boer, University of Amsterdam). The valence changes generally take place at low temperatures, so that in ambient conditions most physical properties, though anomalous, are 'quiet'. The metallic alloys are easier to prepare, are simpler to analyse and are proving a fertile testing ground for the current theories of intermediate valence phenomena.

Preparation of pure and stoichiometric sulphides of rare-earth metals is proving difficult (H. Bach, University of Bochum), especially because of the high volatility of sulphur with respect to the rare-earth metals.

The low-temperature electrical conductivity of SmB₆, and its sample dependence in particular, pose unanswered questions that impinge on the transport theories of disordered systems (Sir Nevill Mott, University of Cambridge). The magnitude of the resistivity of TmS and its relative temperature independence below 1K is a further puzzle. Attempts to extract an effective one-electron theory from the general many-electron formulation, in order to account for the anomalies in lowtemperature specific heat and magnetic susceptibility of the metals, were reviewed by D. Newns (Imperial College). Mixed valence materials are those where one species is present in one of two charge states, and they have already found applications, particularly as dyes. P. Day (University of Oxford) cited many other examples, including Fe₃O₄ (magnetite) and LiTi, O₄ (which goes superconducting).

Other points raised in the open-ended discussion included the inability of the compounds to survive more than ten cycles through the pressure-induced phase transition without damage, and the fact that doping (apart from alloying in metals) has not been investigated, as non-stoichiometry is already providing a wealth of phenomena. Catalytic properties have not been examined but were the subject of speculation.

Given our increased understanding of the semiconducting intermediate valence compounds, it remains for further advances in the methods of preparation to be achieved, and a wider characterization undertaken, before they replace existing materials in device applications or forge a new range of devices based on their unique combination of physical properties.

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^{*}The meeting was held at the Long-Range Research Laboratory at the GEC Hirst Research Centre, East Lane, Wembley, UK, on 15 January 1982

REVIEW ARTICLE

Antibody diversity in lower vertebrates—why is it so restricted?

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Recent studies have revealed that lower vertebrates such as fish, amphibians and reptiles have an antibody repertoire that is much less diverse than that of mammals. It is suggested that the various mechanisms generating antibody diversity are the same in all vertebrates but that non-immunological parameters such as mode of ontogenetic development and cell cycle properties allow fewer somatic events in generating the antibody repertoire of cold-blooded vertebrates.

IMMUNOLOGISTS have always been puzzled by the extent of antibody diversity in mammals, in which the introduction of a single antigenic determinant can stimulate the immune system to produce hundreds of different antibodies. It is difficult to estimate the degree of the huge diversity apparent from these observations: on the one hand counting the genes that encode immunoglobulin gives numbers corresponding roughly to the potential diversity of the system but it is unknown whether these numbers correspond to as many products; on the other hand, by counting products, researchers cannot be sure they are not counting only predominant antibodies, and missing a large number of poorly expressed (therefore undetectable) antibodies. Estimates usually range from 10⁷ to 10⁹ or even more antibodies, speculation in higher vertebrates not being limited by the size of the immune system itself.

We now know that during the ontogenetic development of mouse lymphocytes, various DNA segments assemble and generate a great diversity of genes encoding the variable part of the heavy (H) or light (L) chain of the immunoglobulin molecule. The diversity of the antigen-binding sites resulting from these somatic DNA rearrangements depends on the number of sets and on the number of genes per set. In the most complex case, that of the heavy-chain variable region, the sets V (variable), D (diversity) and J (joining) are assembled to C (the constant-region segment) to form a complete VDJC immunoglobulin heavy-chain gene. There are probably a few hundred V germ-line genes, five J genes and an unknown number of D genes. Since any V gene can assemble apparently with any of the D segments and subsequently to any of the J segments, the diversity generated from this combination is great. It is further amplified by random assembly of the different V heavy chains with light chains to form the complete antigenbinding site. If, in addition, there appear somatic variants due to somatic mutations in the V genes or to joining 'mistakes' during the somatic rearrangement, the potential diversity is further increased (for review see ref. 1). This mechanism, which in mammals uses a large number of inherited germ-line genes and somatic diversification, brings together the once conflicting theories formulated to explain the origin of antibody diversity. However, recent investigations in lower vertebrates indicate that although the antibody repertoire of fish, amphibians, reptiles and perhaps even birds is heterogeneous, it is far less so than in mammals. Two questions immediately arise: (1) does this mean that diversification is achieved by different means in lower vertebrates and in mammals? (2) How can a species survive with such a reduced antibody diversity, or is mammalian diversity necessary? Here I present briefly evidence for reduced heterogeneity of the antibody repertoire in lower species compared with mammals and suggest that the differences are due not only to an evolution of the mechanisms of diversification but also to some non-immunological parameters, such as properties of the cell cycle and the mode of embryonic and larval development.

Low antibody diversity of fishes

Antibody diversity of lower vertebrates has been studied so far by indirect methods based on structural studies, affinity measurements during the immune response, counting isoelectrofocusing (IEF) spectrotypes and by idiotype analysis. No estimate of gene numbers has yet been made in lower vertebrates where immunoglobulin gene analysis is still in its infancy. Some of the methods used are self explanatory: the greater the structural diversity, the number of IEF spectrotypes or the number of idiotypes, the greater the antibody diversity. Affinity measurements have been used to detect an increase in affinity (or maturation of the antibody response). Maturation occurs in mammals and has been interpreted as the consequence of the successive activation of B-cell clones producing different antibodies of increasing affinity. The occurrence of maturation thus implies substantial antibody heterogeneity.

In mammals, a second manifestation of immune response maturation is the successive expression of different isotypes (that is, classes) of immunoglobulin during the immune response. This does not occur in primitive cartilaginous fish, such as the shark Heterodontus francisci, which makes antibodies of the (immunoglobulin M) IgM class only. Both biological and structural data indicate that the repertoire of this species is low. Immunized with 2-furyloxazolone-Brucella, they mount a low-affinity antibody response which varies very little between the different individuals tested and does not increase in affinity after immunization². Although the modified bacteriophage inactivation assay used in these experiments is not ideal for heterogeneity studies because it detects only the predominant antibody in the serum, these results suggest a low antibody heterogeneity both in the species and in individuals; furthermore, the response of this species to a different antigen, pazobenzenearsonate³, has similar characteristics. Limited sequence data on this horned shark immunoglobulin have shown that the variable region appears to be organized in the same way as in higher vertebrates, that is, with a framework and hypervariable regions^{4,5}. The variation between the light chains isolated from different individual animals is, however, very limited, with the major bands having identical isoelectric points. In slight contrast to these results, another species of shark, Ginglymostoma cirratum, immunized with heat-killed pepsinized streptococcal A variant vaccine, produces antibodies that among six outbred individuals have very different light chain gel electrophoresis characteristics⁶. However, as in

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Heterodontus, no increase in variation occurs with time after immunization. Interestingly, in Ginglymostoma the heterogeneity of ligand binding involves intramolecular heterogeneity at the conformational level⁷, meaning that a single molecule could have dual specificities.

Although heterogeneous (that is, more than one antibody per antigen), the anti-dinitrophenol (DNP) response of a bony fish, the carp, consists of IgM antibodies that are all distributed between pH 4 and 6.4 on IEF. The number of IEF spectrotypes (1 spectrotype = 1 antigen-binding band) per individual is small (up to 23) and there is little variation from one outbred individual to the other8. There are always several bands observed per antibody type on IEF gels because of deamidation, and in the absence of a reference fish monoclonal immunoglobulin, it is impossible to say how many antibody types the IEF bands correspond to, but certainly fewer than 23. Moreover, some anti-DNP cross-reactive idiotypes are present at a high frequency in an outbred carp population (in all of 11 immunized animals whereas all preimmune sera were negative)9. This does not necessarily mean that the responses of the bony fish lack specificity: their capacity to recognize two Waldenström protein mammalian idiotypes is similar to that of guinea pigs^{10,11}. If mammalian idiotype diversity were equivalent to immunoglobulin diversity, such a result could mean that the fish repertoire is approximately as large as that of the mammal. However, many mammalian immunoglobulins have cross-reactive or even identical idiotypes and differ elsewhere in the molecule, so that idiotype diversity may be much smaller than immunoglobulin diversity. Moreover, it is unknown whether the fish anti-idiotype antibodies are as heterogeneous as those of the guinea pig.

Amphibian immunoglobulin heterogeneity

In amphibians the primitive urodeles express a very restricted repertoire of antibodies, all of the IgM class as in fish¹². They do not respond well to thymus-dependent antigens¹³, which may be due to a lack of T-cell help but could also be due to the limited repertoire. The fact that there are high levels of natural antibodies to several antigens supports the latter hypothesis: if there are few categories of antibodies, all of them are represented at a high concentration in the serum. More data are available for anurans, particularly Rana and Xenopus. Early work 14-17 on tadpoles proved that anuran larvae could respond specifically (with a number of lymphocytes of the order of 106 cells) to many antigens such as bacteriophages, mammalian red cells, protein antigens and hapten-carrier immunogens like DNP and keyhole limpet haemocyanin (KLH); the maturation of the IgM anti-DNP response was also demonstrated in tadpoles and in adults^{18,19}. Larval and adult frogs responded in a heterogeneous manner, as proved by IEF analysis^{15,20,21}. However, the heterogeneity of the response to red cells, DNP or phosphorylcholine is much more restricted than in mammals. The number of IEF spectrotypes per individual varies from 4 (phosphorylcholine) to 20 (anti-DNP) and very probably corresponds to fewer antibody categories. In the species the number of different anti-DNP antibodies may not exceed 40. These numbers are low compared with those recorded in mammals where, for example, anti-DNP spectrotypes can number as many as 500 (refs 22, 23).

In Xenopus, as in the shark, the amino acid sequence indicates low antibody heterogeneity 24,25 : heterogeneous anti-DNP or even non-immune immunoglobulin pools have provided easily interpretable sequences for the first 16 N-terminal residues of both heavy and light chain variable regions. Altogether, the antibody diversity of anuran amphibians has been estimated at $5 \times 10^4 - 5 \times 10^5$ different antibody molecules.

The availability of clones of *Xenopus* has allowed examination of the role of genetically inherited material in the expression of antibody diversity. Isogeneic *Xenopus* produce antibodies to DNP, xenogeneic red cells, or phosphorylcholine whose IEF spectrotypes are identical or very similar between

isogeneic individuals, whereas they may differ among outbred individuals. The idiotypes of the anti-DNP antibodies cross-react to such an extent that they have been considered identical. Both IEF spectrotypes and idiotypes are inheritable through several generations of cloned animals, suggesting that diversity is not only reduced compared with mammals, but is due essentially to the expression of germ-line genes^{21,25}.

Reptiles and birds

Investigation of antibody diversity has been less extensive in . reptiles and birds. In the former, the lack of or difficulty in detecting an increase in affinity suggests low heterogeneity26 Isoelectric focusing data have suggested a low diversity of antibodies against human serum albumin and DNP in chicken²⁷. Sequence data may also be interpreted in support of this as. chicken light chains seem, on two-dimensional gel electrophoresis, to be less heterogeneous than mouse light chains²⁸⁻³⁰. The poor increase in affinity of chicken antibodies to two haptens (DNP and fluorescyl) may again indicate a lower heterogeneity of these antibodies³¹. However, the antibody repertoire of the chicken to streptococcal A vaccine is as large as that of mice³². In summary, it is difficult to compare frog and bird repertoires, because the only antigen for which heterogeneity has been studied in detail in birds and mammals (the streptococcal A vaccine) has not been analysed in frogs. The possibility remains that birds have an antibody diversity lower than that of mammals.

Discussion

To account for what seems a conspicuous difference between mammalian and lower vertebrate antibody repertoire, I shall first consider the known mechanisms for antibody diversification: (1) increase in diversity of a set of germ-line V, D, J genes by accumulation of germ-line mutations during phylogeny; (2) a mechanism of diversification during ontogeny by combining the various V, D and J elements at random; and (3) a mechanism of diversification by somatic mutation, also during ontogeny.

One may first consider the possibility that in lower vertebrates the immunoglobulin genetic system is simpler than in mammals, for example, that the J or D segments are missing or rather that their homologues are not yet independently duplicated but have remained associated with some part of the variable immunoglobulin gene. These animals may also have fewer genes per category, that is, fewer V, J and D segments.

I suggest that the mechanisms of somatic diversification which operate during ontogeny also occur in lower vertebrates, but that they are influenced by elements outside the immune system, such as cell cycle properties and mode of embryonic development, and that these influences may reduce the effect of one or other somatic diversification mechanism, thereby giving the impression that most antibody diversity is of germ-line origin. Given the great homology of structure between primitive fish IgM and mammalian IgM³³ and the homology between the genes for *Xenopus* and mammalian IgM³⁴, it is likely that immunoglobulin genes in lower vertebrates are organized in a way similar to that of mammals, that is, with a rearrangement of gene segments during lymphocyte ontogeny.

The expression of antibody repertoire can then be influenced by the following. (1) How much wastage of lymphocytes is possible in animals in which lymphocytes proliferate quickly, cell numbers are large, and nonsense mutations can occur without jeopardizing survival; then somatic mechanisms for increasing diversity and especially somatic mutation may be not only tolerated but favoured. This is the case in mammals where lymphocytes can be considered 'cheap'. If cells do not proliferate quickly, if the number of lymphocyte generations is small compared with the life of the individual, or if lymphocytes numbers are small, then the somatic events, even if they occur at the same frequency as in mammals, will have a less important

role. The species will then de facto rely more on its set of germ-line genes and any mutants that may occur among them. This situation occurs in cold-blooded vertebrates, where the cell cycle can be prolonged when temperatures are low³⁵, where cells are often big and hence in smaller numbers, and thus where lymphocytes cannot be wasted and can be considered 'expensive'.

(2) In such species there is even more pressure for the quick expression of a repertoire of germ-line genes because development generally occurs outside the mother and remarkably quickly. If an efficient immune system is not developed within a few days after fertilization, the individuals may be easily infected by external pathogens. Then their immune system has no time to select randomly appearing somatic mutants of lymphocyte clones. It seems likely that their protection will be best ensured by the rapid expression of a selected set of germ-line V genes.

Finally, how can lower vertebrates accommodate a lower antibody diversity and is mammalian diversity a necessity? One may consider that the immune system of lower vertebrates has been submitted to less pressure for diversification because of the existence of non-immunological defence mechanisms which they inherited from invertebrates. There exist in

invertebrates^{36,37} and lower vertebrates³⁸ such mechanisms as natural haemolysin and complement-related compounds which can recognize cell surfaces of bacteria or xenogeneic cells, lyse these cells and hence may prevent the immune system of the animals from seeing many antigens, thereby reducing the external pressure for diversification of antibody repertoire.

Although considerably smaller than those of mammals, the antibody repertoires of lower vertebrates can still be considered very efficient as most antigens can be recognized by a lower vertebrate immune system having a repertoire of possibly at most 5×10^5 antibody varieties. This raises the question of the need for the great diversity of mammalian antibodies. It is not proved that all antibody varieties are necessary, some may be unselected products which could be inherent in any system in which there are somatic rearrangements.

It is also possible that evolution of diversification may move back and forth and there is no indication that the state of antibody diversification known in mammals is the best and final form. We may be observing them in a phase of expansion of antibody diversity preceding a phase of contraction.

The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche and Co.

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Evolution of the Iceland hotspot

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Mantle geochemical enrichment beneath the Iceland-Faeroes hotspot has been episodic, suggesting that blobs rather than a continuous plume may be rising in the region. Recurring rift propagation southward from central Iceland causing progressive melting of the lower crust followed by the upper mantle, best explains the geochemical variations found along the south-west and south-east neovolcanic zones and their degree of thermal maturity.

ALTHOUGH there is no agreement on a particular model for the origin of Iceland, astride the Mid-Atlantic Ridge, it is generally accepted that Iceland represents an anomalously elevated segment of the Mid-Atlantic Ridge where unusual processes and conditions are superimposed on those associated with simple seafloor spreading.

For example, the Iceland crust is thicker and its structure and composition distinct from that of typical oceanic crust¹⁻⁴. The melting zone beneath iceland seems to extend to greater depth, volcanism is more intense, and its products greatly more

varied than along the submerged part of the Mid-Atlantic Ridge².

The composition of the mantle beneath Iceland is apparently also richer in volatiles and radiogenic isotopes of Sr and Pb than the surrounding depleted asthenosphere (that is, the source of normal mid-ocean ridge basalts)²⁻⁷. Some compositional zoning in the mantle about Iceland may also be present^{2-4,8}.

To complicate matters, geological processes by which Iceland developed do not appear to have been constant in intensity nor necessarily continuous in time. For example, palaeomagneto-

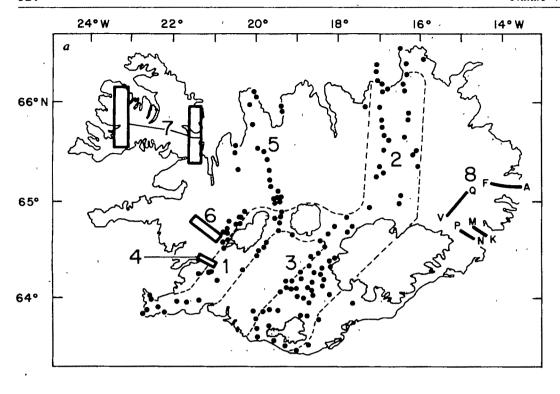
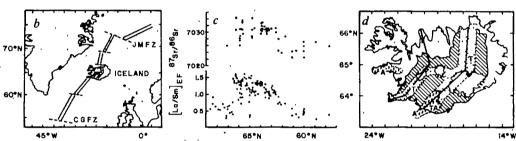


Fig. 1 a, Iceland map, showing location of samples in vol- 12 zones and regions studied: 1, south-west zone (53 samples); 2, north-east zone (37 samples); 3, southeast zone (59 samples), 4, West Iceland (33 samples); 5, Skagi^{18,19}; 6, Borgarfjordur (29 samples); 7, Vestfirdir (68 samples); 8, eastern Iceland (41 samples; letter profiles are from ref 28). b, Iceland and its relation to adjacent Mid-Atlantic Ridge segments. c, Variation of ⁸⁷Sr/⁸⁶Sr and (La/Sm) along the Kolbeinsey Ridge, the north-east and south-west zones and the Reykjanes Ridge. Sr isotope data are from refs 3, 17, 23 d. The distribution of petrological types after refs 35, 36, The jagged line across the south-east transitional alkalı basalts with (La/Sm)>1.75 from tholesites with (La/Sm) < 1.75. Central axes through the volcanic zones used for data projection in Fig 5 are based on the orientation of fissure swarms and volcanic systems^{32 36}

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stratigraphical studies⁹⁻¹¹ and detailed mapping suggest that the volcanic eruption rate has been variable. In addition, the tectonic evolution of Iceland has been complicated by complex rifting zones jumping occurring intermittently throughout its history, sometimes even producing two parallel rifting zones with activities overlapping in time such as the present south-east and south-west neovolcanic zones (Fig. 1)^{1,12-16}. The presence of the East Greenland-Iceland-Faroes Ridge, however, suggests that such unusual activities have been long-lived, and possibly episodic. O'Nions and Pankhurst¹⁷ have also observed a secular decrease in ⁸⁷Sr/⁸⁶Sr in Iceland rocks or age ranging from 16 Myr BP to the present, suggesting that the source of Iceland volcanic products may have varied with time.

Here we review the geochemical data we have accumulated in the Iceland-Faroes-East Greenland region, emphasizing spatial and temporal rare earth (RE) variations in basalts, as well as comparing known Icelandic rift zones at apparently different thermal and tectonic stages of development. For example, the north-east and south-west zones, which are current landward extensions of the Mid-Atlantic Ridge axis (Fig. 1), are considered to be mature rift zones at a quasi-steady state, exhibiting high heat flow and well-developed extensional tectonics1. On the other hand, the south-east zone, a flank zone parallel to the south-west rift zone, appears to be a younger rifting zone in the process of propagating southward. This rift zone does not exhibit any heat flow anomaly and has only poorly developed extensional features¹. It seems to be at a transient stage of development. In contrast, the Skagi zone appears to be an ephemeral palaeorift which developed ~2.5 Myr BP on the flank of the main rift zone in North Iceland, and subsequently died without reaching maturity^{18,19}. In contrast to the south-east zone, there is no petrological and geochemical evidence suggesting that this ephemeral rift developed by propagation but rather evolved uniformly along its entire length.

We will demonstrate that volcanic products associated with rift zones at such distinct stages of development are also petrologically and geochemically distinct. If this interpretation is correct, we can begin to decipher the thermal and tectonic evolution of Iceland in the more remote past.

Our report is based on rare-earth concentrations (La, Sm and Yb) of 334 basalts broadly distributed throughout Iceland (Fig. 1), and isotopic data published^{3,4,17,20-27}. (La/Sm) and (Yb) indicate chondrite normalized enrichment factors for La/Sm and Yb. A good age control for Pleistocene-Miocene lavas was obtained in eastern Iceland, Vestfirdir, Borgarfjordur and West Iceland by coordinating our sampling with existing age dating and palaeomagnetostratigraphic studies of these regions^{9-11,28-30}. Part of the RE data has been published elsewhere^{2,19}, and the remaining RE analyses can be found in ref. 31.

Temporal variations

Rare-earth patterns of all the Iceland basalts studied are shown in Fig. 2a. There is an important difference in the type and range of pattern for Pleistocene-Miocene (13.5-0.7 Myr) basalts and basalts from the neovolcanic zones (<0.7 Myr). Pleistocene-Miocene lavas are consistently enriched in light-RE, whereas more recent lavas exhibit a wider spread in light-RE from enriched to depleted patterns more like those of normal mid-ocean ridges. The (La/Sm) distribution in Pleistocene-Miocene lavas is normal with a mode around 1.65

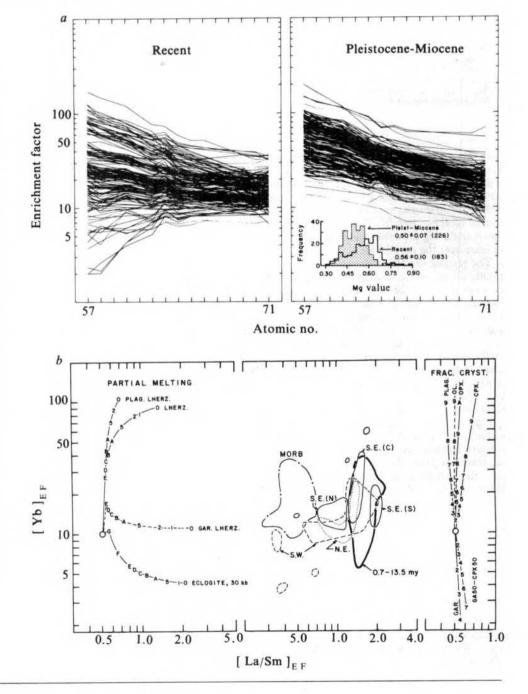
and an average of 1.7 ± 0.27 (1 s.d.), whereas the (La/Sm) distribution in Recent lavas tends to be positively skewed with a mode around 1.35 and an average of 1.34 ± 0.40 . There is also a significant statistical difference at the 99% confidence level in the distribution of Mg-value of Pleistocene–Miocene lavas which is normal with a mode around 0.5, and that of Recent basalts which has a mode around 0.6 and is positively skewed (Fig. 2a). Figure 3 shows that light RE-depleted basalts $\{(\text{La/Sm}) < 1\}$ occurring in Recent time are confined to basalts high in Mg-value (Mg/Mg+Fe²⁺, atomic concentration ratio) representing either olivine tholeiites or picritic basalts as also found by other authors^{18,19,25}. Finally, the new Miocene picritic basalts we have encountered in Vestfirdir are light-RE enriched and not depleted as in Recent time. Thus, light-RE depleted basalts seem to reflect eruption of a basalt type which has clearly developed on Iceland only in Recent time.

The above results suggest a secular decrease in light-RE enrichment from Tertiary to Present which is best illustrated in the Eastern Plateau basalt succession (Fig. 4). A broad regular decrease is readily noted from 13.5 to 2 Myr. Extrapolation of this trend below 2 Myr BP to the zero age intercept coincides

with the mean value found for the north-east neovolcanic zone, which is the locus of the present spreading axis in northern Iceland. The secular variation extends even within the northeast zone where the dormant east fissure swarm is dominated by light-RE enriched basalts, whereas light-RE depleted patterns are more predominant in the now active western fissure swarm mapped by Walker³². Thus this is also apparent in West Iceland between basalts greater and <0.7 Myr BP. The rate of light-RE decrease seems to have increased significantly within the past 0.7 Myr BP.

In addition to the secular decrease in (La/Sm), there is a concomitant secular decrease in ⁸⁷Sr/⁸⁶Sr throughout Iceland suggesting that the mantle source of these basalts beneath Iceland has evolved¹⁷. Such secular geochemical variations are not unique to the past 14 Myr history of the Faeroes-Iceland-Greenland Ridge, but has also been observed during the later period of the Faeroes Island Plateau basalt formation some 55–50 Myr BP, at the onset of continental drift in this region of the North Atlantic^{26,33}. (La/Sm) and ⁸⁷Sr/⁸⁶Sr decrease and ¹⁴³Nd/¹⁴⁴Nd increases as one proceeds from the lower and middle basalt series of the Faeroes^{26,31}. The Plateau basalts

Fig. 2 a, Rare-earth patterns for Recent basalts (<0.7 Myr BP) Pleistocene to Miocene basalts (0.7-13.5 Myr BP). Enrichment factor refers to REE concentration relative to chondrites. Histograms compare the distribution of Mg-values corresponding to these two populations. b, (Yb) against (La/Sm) variation for Iceland basalts compared with theoretically derived RE generated by variations degrees of partial melting (batch melting) of various mantle sources (left) and different degrees of fractional crystalliz-ation (right). The field for Pleistocene-Miocene basalts (solid line labelled 0.7-13.5 Myr) is based on data from the Eastern Iceland Plateau, Vestfirdir, Borgarfjordur and West Iceland basalts >0.7 Myr old. SE (N), SE (C) and SE (S), northern, central and southern part of the south-east zone, respectively. SW, south-west zone; NE, north-east zone. MORB, normal mid-ocean ridge basalts from the Kolbeinsey and Reykjanes Ridges. The starting compo-sition for partial melting and fractional crystallization models was arbitrarily set at (Yb) = 10 and (La/Sm) = 0.5. Plag., lherz., lherz., gar. lherz., and eclogite plagioclase lherzolite source plagioclase. 48% olivine. orthopyroxene, 16% clinopyroxene), a lherzolite source (60% Ol, 20% Opx, 20% Cpx), a garnet lherzolite source (48% Ol, 16% Opx, 16% Cpx, 20%) and an eclogite source (50% Cpx, 50% Gar). 0, 2, 5 and A, B, C, D, E, F, G along the partial melting curves indicate 0, 2, 5, 10, 15, 20, 25, 30, 50 and 77% melting. 2, 3, 4, 5, 6, 7, 8, 9, A along the fractional crystallization curves indicate 20, 30, 40, 50, 60, 70, 80, 90 and crystallization of plagioclase (Plag), olivine (OI), orthopyroxene (Opx), clinopyroxene (Cpx), garnet (Gar) and an eclogitic mixture (Ga 50-Cpx 50). Partition coefficients used are as given in ref. 71.



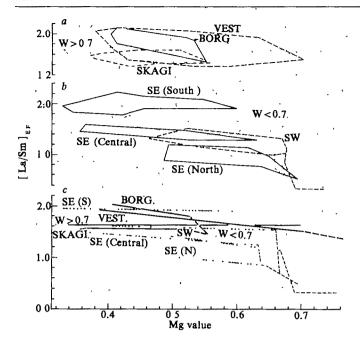


Fig. 3 Co-variation between Mg-value and (La/Sm) for individual zones. a, Variations in the Pleistocene-Miocene zones; Vestfirdir (VEST.), Borgarfjordur (BORG), Skagi and West Iceland (W) > 0.7 Myr. b, Variations in the Recent zones; south-west (SW), south-east (SE) and West Iceland (W), 0.7 Myr. c, Variations in the Recent zones; south-west (SW), south-east (SE) and West Iceland (W), 0.7 Myr. c, Miocene zones (bold solid lines).

from East Greenland's continental border are similar to the Faeroes upper series, suggesting symmetry about the Mid-Atlantic spreading axis³³. We conclude that the secular (La/Sm) observed both in eastern Iceland, and earlier in the Faeroes, reflect a true secular variation in the composition of the mantle source of these basalts. The enrichments seem to have been episodic during the evolution of the Iceland hotspot evolution at least over the past 60 Myr.

The secular variations shown in eastern Iceland may not be totally devoid of complexity due to spatial variations (Fig. 4). A close look at the broad (La/Sm) secular decrease, may indicate a variation of a shorter wavelength, with a maximum around the 7-8 Myr period. Such a perturbation may be significant as it coincides with a period of high lava extrusion rate^{9,11} and the proposed sudden increase in volcanic activity on the Reykjanes Ridge which apparently initiated the development of time transgressive V-shaped ridges³⁴. However, there is also a 60-km break in the sample profile at this point, and thus the perturbation could also reflect some small but significant spatial variations existing at that time.

Figure 4 further shows that the regular secular trend noted in the Eastern Iceland Plateau is generally not corroborated in western Iceland based on composite basalt profiles from widely separated regions, though basalts from Vestfirdir are statistically indistinguishable from the Eastern Plateau basalts over the same 13.5-9 Myr BP period. This may partly be due to our inadequate sampling which does not permit us to take into consideration possible Tertiary spatial variations along rift strikes, such as those currently found along the neovolcanic zones, and partly due to complications imposed by rift jumping and the probable presence of parallel ephemeral rift zones such as Skagi and possibly Borgarfjordur.

Spatial variations

For practical reasons, our study of spatial variations across Iceland has been limited to known palaeo- or neovolcanic rift zones. We have projected the variation in (La/Sm) and Mg-value within these zones along two major profiles running

essentially parallel across Iceland (Fig. 1): the Eastern and Western Profiles (Fig. 5). Note that all the lavas from the Eastern Profile are essentially contemporaneous, whereas there is an age discontinuity on the Western Profile corresponding to Skagi (0.5-2.5 Myr) and the south-west zone (<0.7 Myr). Clearly there are important RE and Mg-value variations between the neovolcanic zones as well as within them. (La/Sm) and Mg-value respectively decrease and increase northward. along both the south-east and the wouth-west zone, but at a faster rate in the SW zone. On the other hand, 87 Sr/86 Sr appears relatively constant along the entire south-west zone³, but tends to increase southward along the south-east zone^{3,20,21,23,27}. The increase in (La/Sm) along the south-east zone is rather stepwise, and the variation essentially reflects three major petrological zones recognized first by Jakobsson (Fig. 1)^{35,36}. This is not the case along the south-west zone which shows no petrological gradient and is dominated by olivine tholeiites and tholeiites³ In contrast, the (La/Sm) and Mg-value variations in the Skagi palaeorift zone and north-east neovolcanic zone show no well developed spatial trends along strike^{18,19}, although there is a lateral difference in the distribution of RE patterns between the dormant western and the active eastern fissure swarms of the north-east zone32.

Finally, our extensive survey demonstrates that the strongly light-RE depleted picritic basalts previously reported in Central Iceland^{20,23,27} and the Reykjanes Peninsula³⁷ are, in fact, limited to these two regions which have been proposed to be fracture zones^{1,18} (Fig. 5). None were found elsewhere along the neovolcanic zones nor in the Pleistocene-Miocene lavas of Iceland. Only three picritic basalts were observed in our survey of Pleistocene-Miocene lavas, but these have light-RE enriched patterns. The spatial contrast within and between known rift zones of Iceland is further illustrated in (La/Sm) versus Mgvalue plots (Fig. 3). The (La/Sm) appears to be insensitive to

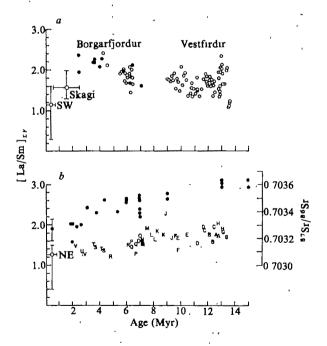


Fig. 4 Secular variation of (La/Sm) through the basalt plateaus in western (a) and eastern (b) Iceland. In a, \bullet , samples dated by the K-Ar method¹⁰; C, samples whose age has been estimated from their stratigraphical height and the assumed linear relationship between stratigraphical height and age from ref. 10 for Borgarfjordur and McDougall and Kristjansson (in preparation) for Vestfirdir. Ages between 13.5 and 10.5 Myr are based on the average K-Ar ages of several profiles while ages between 10.5 and 9.0 Myr are based entirely on field relations relative to older, dated lavas The average (La/Sm) for the south-west (SW), and Skagi zones are shown for comparison, error bars corresponding to the range in age and (La/Sm) observed in each zone In the eastern Iceland series, letters refer to different profiles (see Fig. 1). Ages of samples in this series are based on the palaeomagnetic stratigraphy in ref. 11 The average (La/Sm) in the northeast (NE) zone is also shown. 87 Sr/ 86 Sr data (\bullet) is from ref. 17.

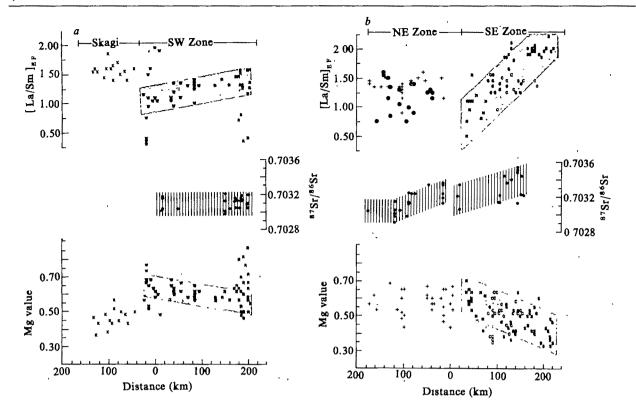


Fig. 5 (La/Sm), Mg-value and ⁸⁷Sr/⁸⁶Sr variations along. a, western (SW and Skagi), b, eastern (SE and NE) volcanic zones. Distance along the abscissa refers to distance away from central Iceland 64.86° N) along the axes of the volcanic zones (see inset Fig. 1). Sample locations have been projected normally to zone axes. Samples from the active western ● and dormant eastern + part of the north-east zone are indicated. Mg-value data for the north-east zone is from ref.

72, whereas the ⁸⁷Sr/⁸⁶Sr variations were constructured from data in refs 3, 4, 17, 20-25 and 27.

Mg-value variation in a particular zone, but the level of (La/Sm) is distinct from one zone to another.

We consider that the distinct (La/Sm) level within each region reflects similar mantle source variations. On the other hand, the different (La/Sm) levels observed within the south-east zone, which also corresponds to three different petrological types^{35,36}, suggest both distinct sources as well as melting conditions.

Model constraints

Although numerous models have been proposed to explain trace element and isotopic variations in isolated regions in Iceland (see refs 22, 23, 25, 38–40), few have been suggested for the entire Iceland plateau and surrounding ridge segments that account for both spatial and temporal variations in a unified way.

In general, processes which have been suggested to affect RE patterns in Iceland basalts range from: (1) fractional crystallization; (2) partial melting conditions in the mantle (such as degrees of melting, mineralogy and dynamics of melt removal); (3) magma-wall rock interaction; (4) zeolitization and spatial variations within eruptive lenses^{41,42}; (5) mixing of different magmas or reservoirs; (6) mantle heterogeneities (of specified or unspecified causes).

Merits of these possibilities are briefly evaluated before we propose our preferred model.

(1) Although it is clear that fractional crystallization took place from the presence of phenocrysts, large (Yb) variation and Mg-value variation within individual zones (Fig. 2b and Fig. 5), such a process cannot account for the (La/Sm) variation observed within a particular region. This is also ruled out in any region where there is a covariation of (La/Sm) and ⁸⁷Sr/⁸⁶Sr, assuming that equilibrium rather than disequilibrium partial melting takes place (Figs 4 and 5).

(2) Variation in degree of batch partial melting is also unlikely to account for the secular variation in RE patterns noted in Figs 2a and 4 as the range of partial melting would be from a few per cent to 100%, and thus is untenable petrologically speaking, considering that the mantle is most probably peridotitic. Of course, some variation in partial melting is likely to take place and is permissible depending on the existing thermal regime, type of lava erupted, and isotopic ratios observed²⁷. The south-west zone is an example where (La/Sm) decreases towards Central Iceland, whereas ⁸⁷Sr/⁸⁶Sr remains essentially constant (Fig. 5), and the south-east zone is an example where ⁸⁷Sr/⁸⁶Sr and (La/Sm) co-vary and the petrological basalt type changes from tholeitic to alkalic (Fig. 5).

Wood's model⁴³ invoking fractional melting is also unlikely to explain the secular variation observed for both the RE and ⁸⁷Sr/⁸⁶Sr (or ¹⁴Nd/¹⁴⁴Nd). It is sufficient to say that the build-up of 87Sr/86Sr with time is irreversible. Once a source region high in 87Sr/86Sr has been established, successive partial melting of such a source region in equilibrium conditions will not decrease this ratio (which has been corrected for any possible isotopic mass fractionation, see ref. 3). It is also improbable that models which consider disequilibrium melting of a veined mantle^{27,44} can explain the observed secular trend over approximately a 10-Myr period of seafloor spreading and the cyclic nature of the Iceland hotspot during the past 60 Myr. This would require a mechanical decoupling of the crust generated by spreading and the mantle source from which melts are derived (that is, decoupling of the crust from the lower part of the lithosphere). This is totally inconsistent with plate tectonic theory, structure of the lithosphere and the distribution of earthquakes. Furthermore, application of the concept of such disequilibrium melting⁴³ for generation of basalts, which seem to have required rather large degrees of melting, does not seem viable on the basis of Hofmann and Hart's⁴⁵ kinetic and scaling analysis.

(3) It is difficult to imagine how magma-wall rock interaction could account for the regular spatial and temporal variation

and cyclic nature of the geochemical variation noted. Indeed, because very *ad hoc* conditions would have to be invoked we reject this possibility.

(4) Wood and co-workers⁴⁰⁻⁴² interpreted the RE variations observed in eastern Iceland in terms of spatial variations within eruptive lenses rather than temporal effects. Wood⁴¹ also suggested that the high (La/Sm) found in the oldest basalts was due to increasing depth or burial and zeolitization of the lava pile. We find Wood's explanations unlikely because: first, basalts of the same age from the Vestfirdir Plateau (9-13.5 Myr), which we have studied because they have not been zeolitized, also have high (La/Sm), indistinguishable from that found in basalts of the same age from eastern Iceland (Fig. 4). Second, Miocene picritic basalts from Vestfirdir are light-RE enriched and not depleted, as Wood suggests for high MgO basalts from shield volcanoes erupted near lens margins, which would have subsequently been eroded⁴². Third, the secular variation shown in Fig. 4 using the new dating scale of Watkins and Walker¹¹ is much smoother than that observed using the scale of Dagley et al.²⁸. This supports our view that the variation is indeed time-related, rather than spatial^{41,42}. Fourth, contrary to Wood's model predicting increasing (La/Sm) with increasing depth of burial and grade of zeolitization; our samples from both the N section within the highest grade laumantite facies and sections J to P in the lower grade mesolite facies fall on the general secular trend shown in Fig. 4, and not above; our basalts from sections A and B which are of similar age and geographical location have similar (La/Sm) despite the fact that section A has undergone a higher degree of metamorphism (mesolite facies) than section B (analcite facies) (Fig. 4); and

this is also the case for sections F and J. Finally, in addition to the secular decrease in (La/Sm), there is a concomitant decrease in ⁸⁷Sr/⁸⁶Sr and an increase in ¹⁴³Nd/¹⁴⁴Nd both on Iceland during (the past 13 Myr (refs 17, 24) and on the Faeroes 60–50 Myr BP (ref. 26). This is too much of a coincidence to be attributed to secondary alteration⁴¹, as for both Iceland and the Faeroes, the (La/Sm) and isotopic trends were established on totally different sample suites. Furthermore, the radiogenic Nd isotopic ratio is unaffected by weathering and mild metamorphism or metasomatism⁴⁶.

(5) Oskarsson et al.⁴⁷ have proposed a petrochemical model for rift zones on Iceland which is consistent with the crustal accretion model of Palmason⁴⁸. Basalt variations are explained by mixing between mantle derived olivine-tholeiites and variable amounts of crustal-derived magmas ranging from silicic to nepheline-normative melts. Flank zone volcanism^{35,36,38} is considered to be dominated by crustal-derived magmas, whereas the main axial rift zones are dominated by mantle-derived olivine-tholeiites (Fig. 1b). A crucial assumption of this model is that the subsiding crust becomes completely hydrated by hydrothermal activity. Studies of DSDP rocks of various ages in the oceans, show that hydration of the oceanic upper crust is very irregular and incomplete, despite the ample abundance of seawater above. It is doubtful that on Iceland where such an abundant source of fluids is absent, complete hydration would in fact take place. Their model also does not consider the temporal, nor spatial, variations found along the main axial zones discussed here. However, magmas of crustal derivation in the southern part of the south-east zone is an attractive

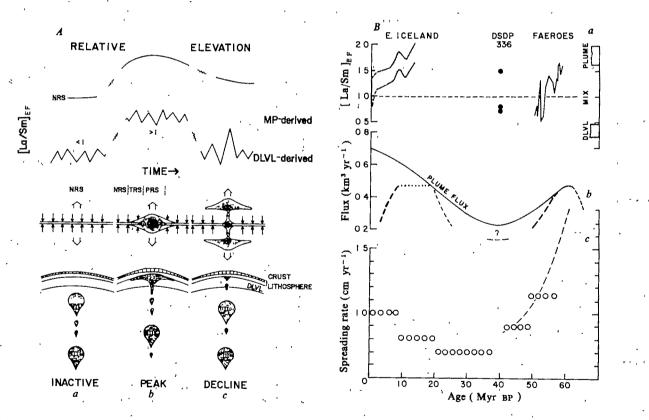


Fig. 6 A, model of a mantle plume (MP) ridge system showing variation in plume flux with time and a cycle in plume (blob) activity that is consistent with geochemical variations observed in erupted basalts. Phases. a, plume is rising beneath an existing spreading ridge (passive spreading); b, plume-flux exceeds the gap created by spreading resulting in blob flattening and spill along spreading directions (active spreading). Plume-derived material dominates the erupted products which accumulate as plateau basalts; c, tailing phase of the blob. Plume flux is insufficient to feed the overlying spreading ridge and material from the depleted low velocity layer (DLVL) compensates Small arrows indicate horizontal component of asthenospheric flow of DLVL material toward the ridge axis. Large arrows indicate spreading directions of the overlying lithospheric plates NRS, TRS and PRS stand for normal, transitional and plume ridge segments B, Reykjanes Ridge spreading rates, Faeroes-Iceland mantle plume flux and (La/Sm) variations with time modified from ref. 33 Curves c are spreading rates by Vogt and Avery. (dotted-dashed curve) and by Herron and Talwani⁵¹ (O). Curve b is the plume flux (solid line) estimated from Vogt and Avery, assuming basalts represent 25% partial melting and, therefore, that basaltic discharge is equal to one-quarter of the total plume flux. The dashed lines indicate our estimate of plume flux for the Iceland-Faeroe plume during the 60-50 (Schilling and Noe-Nygaard 1974) and 13.5-0 Myr periods, based on (La/Sm) as shown in A.

• Data from DSDP site 336 (ref. 51)

hypothesis but the crust need not be hydrated to the extent indicated by these authors.

Preferred model

The binary mixing asthenosphere-plume or blob model proposed earlier (Fig. 6)³³ can readily explain the noted temporal and spatial co-variation of (La/Sm) and ⁸⁷Sr/⁸⁶Sr, but is inadequate when there is a decoupling between these two parameters, such as in the south-west zone (Fig. 5). It also fails to explain readily the southward (La/Sm) and ⁸⁷Sr/⁸⁶Sr decrease along the SE zone since it is of opposite sense to the gradient observed along the Reykjanes Ridge and Peninsula. We propose a composite dynamical model. The blob model explains the secular variation whereas spatial variation such as, for example, along the south-east zone is explained by rift propagation into older crust, resulting in, first, melting of the older large-ion-lithophile-enriched (LILE) lower crust-lithosphere, the enrichment reflecting an earlier stage in blob activity, followed by melting of the upper mantle due to advection.

In the blob model, the RE pattern of a basalt primarily reflects the mixing proportions between a light RE-enriched mantle plume source ({La/Sm}>1(and the asthenosphere which is characteristically depleted in light RE ({La/Sm} \le 0.6) (Fig. 6). The degree of mixing depends on the interplay between the rate of mantle plume upwelling and the rate of lithospheric plate divergence above the upwelling region, both of which are allowed to vary independently through time³³. Figure 6b compares the known temporal variation for the Iceland-Faeroe Ridge with the concurrent variation in spreading rate of the Mid-Atlantic Ridge, and a mantle plume flux, modified from Vogt and Avery's 49 curve for rate of basalt discharge in accordance with our new results. The (La/Sm) decline in the 50-60 Myr period could not be accounted for by a change of spreading rate as spreading would have had to increase rather than decrease³³. Therefore, the (La/Sm) decline must reflect a decrease in plume flux³³. Interpretation of the (La/Sm) secular decline within the past 13 Myr BP in Iceland depends on how reliable the spreading rate estimates are during this period (Fig. 6b)49,50. At face value, the (La/Sm) decrease during the 9-13 Myr period could be accounted for by increasing spreading, but not during the past 9 Myr which again would require a decline in plume flux which would be particularly severe within the past million years. The blob model further suggests that we are witnessing in Iceland the declining phase of mantle blob activity, that is, a rapid change to a more passive mid-ocean ridge type spreading regime. If such a trend is extrapolated into the future, the model predicts either a change from a subaerial to a submarine regime and the possible split of Iceland into two islands located symmetrically about the Mid-Atlantic Ridge, or a new phase of plateau basalt formation, depending on the periodicity and topology of the proposed blobs.

It has been suggested²⁷ that the large (La/Sm) variation observed in successive flows sampled in DSDP Hole 336 on the northern flank of the Faeroes-Iceland Ridge⁵¹, may not support the proposed cycle (Fig. 6). We contend that what happens in the interim period between two blobs depends on the exact topology of the blob tail, that is the nature of schlierens trailing behind the main body of the blob (see ref. 52). Such interim periods may be characterized by inefficient mixing and thus possible alternation of lava with the geochemical signature of one source or the other. Rapid fluctuation between light-RE enriched, and depleted patterns is also observed on the Reykjanes Peninsula^{25,37} as well as in the later phase of volcanism on the Faeroes just before the spreading began.

The blob-asthenosphere mixing model is supported by the detailed nature of the RE patterns shown in Fig. 2b. There is a (La/Sm) gap between <0.3 and 0.4 which corresponds to the field occupied by normal ridge segments adjacent to Iceland⁸. (La/Sm) variations on the right of this gap are explained by mixing the blob and depleted asthenospheric material whereas

to the left fractional melting is probably the cause of such unusually light-RE depleted picritic basalts which seem to occur only in Recent time, as previously suggested 19,43,44.

We further propose that the spatial variation in RE patterns and petrological basalt types found along the recognized neovolcanic and palaeorift zones of Iceland seems to depend on the degree of thermal and tensional tectonic maturity of the zones and existing mantle source heterogeneities beneath them. The northward progressive decrease in (La/Sm) and the increase in Mg-value observed along both the south-west and south-east zones, but at a faster rate in the south-east zone (Fig. 5), are best explained by a temporal change in intensity and conditions of melting resulting from rift propagation. For example, the south-west zone, a direct extension of the Mid-Atlantic Ridge exhibits high heat flow and well developed extensional tectonics¹ which we interpret as reflecting maturity¹9, whereas such features are lacking in the south-east¹, thus suggesting youth.

The thermal process resulting from such rift propagation is best described in two stages, though in reality it probably evolves more continuously. We assume first that crustal tension released at the tip of the rift propagator evolving along the south-east zone is sufficient to bring the older and colder late Miocene lower crust (~8 Myr old) into the partial melting stability field, resulting in magmas of crustal origin (and perhaps old lithosphere as well) with Sr and Nd isotopic signatures of late Miocene lavas exposed elsewhere on Iceland 17,20,24. RE patterns will also be light-RE enriched, but detailed modelling is required to take into account the possible mineralogical composition of the lower crust (and old lithosphere), degree of melting and type of lavas generated31. As spreading develops and the rift matures, there is a progressive change from primarily crustal-derived products to basaltic melts derived from mantle advection. We have seen earlier that the mantle source on Iceland in Recent time, particularly within the past 700,000 yr has become significantly diluted with light-RE depleted asthenospheric material. Thus, basalt erupted in a later stage, far beyond the tip of the rift propagator, will also be lower in ⁸⁷Sr/⁸⁶Sr, higher in ¹⁴³Nd/¹⁴⁴Nd (ref. 24), and less light-RE enriched. In other words, in this model the present spatial variation observed along the south-east zone is, in fact, the result of a temporal evolution in melting intensity and conditions, due to rift propagation. Melts erupting near the tip of the propagator are primarily derived from the older late Miocene lower crust-lithosphere, whereas in the northern part, as rifting and melting increase, basalts are derived from a mantle plume-asthenospheric mix, now dominated by the latter type of material. Increasing melting towards the centre of the plume (central Iceland) is supported by a concomitant increase in ²³⁰Th /²³²Th /²³²Th /²³⁴ 523 Th Th/232Th (ref. 53) and an increase in volcanic delivery rate which is manifested by a widening of the rift zones and a greater abundance of central volcanoes 13,35,36. The tip of the present rift propagator along the south-east zone probably lies just south of Surtesy Island, which emerged in 1963. Whether such a propagating rift is of the membrane type⁵⁴ as suggested for the Vestmannaeyjar-Surtsey Zone and the Settla Peninsula55, or of Hey's type⁵⁶ is uncertain.

We also suggest that the south-west zone may also have developed by rift propagation, but earlier relative to the south-east zone, that is some 6 Myr BP (ref. 57). This is indicated by: (1) the similarity but different intensity of the gradient in (La/Sm) and Mg-value between the two zones (Fig. 5); (2) the presence of a well developed heat flow pattern and extensional tectonics in the mature south-west zone and their absence in the south-east zone¹; and (3) the presence of a ⁸⁷Sr/⁸⁶Sr gradient along the south-east zone but its absence in the more mature south-west zone (Fig. 5). Recent basalts erupted along the more mature south-west zone are all derived from the upper mantle composed of a blend of LILE-enriched blob and LILE-depleted asthenospheric material, with the degree of melting increasing slightly towards the centre of the plume (central Iceland) to account for the small northward decrease in (La/Sm) (Fig. 5)¹⁹.

If our assumptions concerning petrological and geochemical types and degrees of maturity of neovolcanic rift zones are correct and applicable to the more remote past, the following suggestion can be made on the nature of rifting throughout Iceland's history. Generation of the Eastern Plateau and the north-east zone lavas during the past 13 Myr seems to have been associated with continuous spreading and decreasing blob flux into the source region, and has been accompanied by flank rifting activity at least once in the past along the Skagi zone some 2.5-0.7 Myr BP. However, the petrological and geochemical uniformity of basalt erupted along the Skagi zone 18,19 precludes that it developed by rift propagation. Thus, so far there is no evidence for rift propagation in northern Iceland. In contrast, the tectonic evolution of southern Iceland has been complicated by recurring southward propagation of rifts of ephemeral duration and simultaneous flank zone volcanism, as evident in the Borgarfjordur region (7-2 Myr), the south-east zone and the Setberg volcanic region on the Snaefellsnes Peninsula⁵⁸. This evolution is generally consistent with the tectonic evolution models proposed for Iceland 12,14,57. However, there are many uncertainties and several discrepancies which cannot be discussed further without more extensive field work in coordination with additional rock dating and geochemical and petrological studies.

Problems

Although our new data on Iceland support the blob model for the evolution of the Iceland-Faeroes hotspot, the genesis of such enriched blobs remains uncertain. The fact that such mantle material is enriched in the radioactive heat-producing elements (U, Th and K) could suggest a model by which internal heating of a deeper mantle layer rich in these elements would cause the layer to become gravitationally unstable and to rise in the form of blobs^{3,52}. Another possibility is the recent model involving lithosphere recycling⁵⁹. However, this is not supported by the high ³He/⁴He ratio of lavas near and on Iceland^{60,61}. The fact that such anomalous mantle regions are also richer in volatiles^{5,7,62} has also led to the suggestion that metasomatism of the upper mantle^{25,41,62,63}, perhaps resulting from fluids released from deeper mantle convection⁶², has produced the observed enrichments. In the latter case, interstitial fluids rising through the mantle and sweeping out large incompatible ele-

Received 11 September 1981, accepted 25 January 1982

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ments, accumulate in the upper mantle below the lithosphere. Such volatile enrichment would also enhance melting by lowering the melting point of the upper mantle and account for the greater volcanic flux in such regions. Application of this model to the Iceland-Faeroe Mid-Atlantic Ridge System would require that such a metasomatic front be geographically localized, spatially zoned, and episodic.

The above possible plume models are not necessarily exclusive. In fact, there is growing evidence that different plumes have different chemical and isotopic signatures and possibly different origins^{8,64}. Some may be related to lithosphere or crustal recycling; others may have a more primary origin^{60,61}.

Another point, inherent of the method of using lavas to constrain possible mantle flow geometries, is whether the gradients found along the Reykjanes Ridge²⁻⁴ reflect a similar zoning of the underlying mantle, or if they reflect a dynamical mixing condition of plume with depleted asthenospheric derived material during generation of new lithosphere². Distinction between these two possibilities would facilitate the choice between the plume or blob model and the metasomatic model. The difficulty lies in the fact that we do not know from what volume and geometry (topology) of the mantle a lava flow erupted on the Earth surface is derived from, has equilibrated with and thus is compositionally representative of. As an example of two possible extreme cases, we ask, is magma along the ridge axis derived primarily from a vertically deep and narrow column of mantle, or alternatively, laterally from a relatively thin mantle layer? Large lateral movement of magmas^{2,65,66} along strike during dyke propagation is also likely to add further interpretative complications.

Until these two major problems are resolved little advance can be made in choosing between the blob and metasomoatic models, or other models including the veined mantle, or vertically zoned mantles of one type or another (see refs 67-69).

A more detailed discussion of the data can be found elsewhere^{31,70}.

This work was partly supported by NSF grants DES75-03997 and EAR77-23736 to J.-G.S. and H. Sigurdsson. Field work and petrological studies were carried out in collaboration with H. Sigurdsson. We thank Rannsoknarad Rikisins for permission to collect samples in Iceland, also Frank DiMeglio, Mike Doyle and staff of the Rhode Island Nuclear Center for their assistance in neutron activation analyses, and Gloria Nappi for assistance.

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Processed genes: a dispersed human immunoglobulin gene bearing evidence of RNA-type processing

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A dispersed immunoglobulin pseudogene carries two hallmarks of RNA processing—spliced J and C regions and a poly (A)-rich tail. Its discovery strengthens the notion that processed genes are a significant feature of the mammalian genome and that genetic information can return to the genome via an RNA intermediate.

ONE of the α -like globin genes of the mouse has undergone two curious rearrangements; it has cleanly lost both its intervening sequences^{1,2} and it has moved from the active α -globin locus to another chromosome^{3,4}. This association of gene movement and precise splicing suggests that an RNA intermediate might have been involved in the formation of this novel gene1 as well as in its conveyance to a new location3.5. The additional finding of multiple, distantly related members of the globin gene family3 further suggests that this gene is not an isolated example, but rather that dispersed and possibly spliced genesor 'processed genes' as we shall refer to them-represent a significant class of genetic element and are the result of a major mechanism of genomic evolution. (By processed genes we refer to gene-like sequences that, as opposed to their normal counterparts, bear some evidence of RNA-type processing; for example, coincident homology to the site of transcriptional initiation, clean loss of intervening sequences or coincident homology to the site of poly(A) addition, possibly including elements of a poly(A) tail.)

Here we describe a human gene sequence that fulfills these expectations in a particularly interesting way. The gene is a pseudogene corresponding to the human immunoglobulin λ chain, the major locus of which is on human chromosome 22 (refs 6, 7) wherein the normal gene is represented in at least six non-allelic copies⁸. This pseudogene, $\lambda \psi 1$, is no longer at this locus, but has been conveyed to another chromosome. Here it is no longer represented as discontinuous J- and C-region segments, but rather the J and C regions are cleanly joined, again in accordance with the rules of RNA splicing. The gene bears an additional hallmark of RNA processing; its homology to the normal immunoglobulin gene ends abruptly in a long sequence of adenylic acid residues that resembles a poly(A) tail. These facts, in addition to the curious state of the 5' ends of this and the α -globin pseudogene, lead to a minimal—though entirely speculative-model that invokes an RNA intermediate in their formation and conveyance.

Identification and cloning of the λ -like genes

Our previous studies of the immunoglobulin λ light-chain genes of man had shown them to be arranged within a complex locus consisting of six constant region (and presumably at least an equal number of J region) genes, arrayed along a \sim 45-kilobase (kb) stretch of DNA on human chromosome 22 (ref. 8). When cleaved with the restriction endonuclease EcoRI, these genes fall into three fragments, usually 16, 14 and 8 kb long. The smallest (8-kb) fragment occasionally exists in one of several polymorphic forms, the most prevalent one in populations we have analysed being 18 kb. Several other more faintly

hybridizing fragments can also be identified by high-resolution gel electrophoretic analysis or by direct gene cloning. These include a large (>20 kb) fragment, an 18-kb fragment that underlies the strongly hybridizing 18-kb polymorphic fragment, a 16-kb fragment and a small 5-kb fragment that is evident in all human samples analysed. We have also cloned an additional, strongly hybridizing 14-kb fragment that has not yet been linked to the major locus.

The complexity of the human λ locus and its accompanying array of faintly cross-hybridizing gene fragments is reminiscent of the situation encountered among the mouse globin genes^{1-4,9-11}. The more distant relationship between the active λ genes and their structural relatives encoded on the >20- and 5-kb fragments is shown by high and low stringency in in situ hybridization experiments in which cloned active λ constantregion genes and a faintly hybridizing clone, $\lambda \psi 1$, are hybridized to total human genomic DNA (Fig. 1). When active gene sequences (genes 1 and 2 in Fig. 1, corresponding to the Mcg and Kern Oz genes) were used as probes, four strongly hybridizing bands were seen. Each corresponds to the four fragments that can be derived from the active locus of an individual polymorphic for both the 8- and 18-kb active fragments. In addition, faintly hybridizing >20- and 5-kb bands can be visualized in these high stringency conditions, and appear as more intensely hybridizing bands when the stringency of the washing conditions is reduced from 52 to 45 °C. The relative intensities of the >20- and 5-kb bands further suggest that the latter band is more closely related in sequence to the active genes than is the sequence encoded on the larger band (although it is also possible that the larger band is transferred less efficiently to the blotting paper or has a smaller segment of homology).

In any case, these blotting and cloning experiments, coupled with our inability to link the cross-hybridizing >20-, 18-, 16-, 14- and 5-kb fragments to the active locus through map extension strategies using cloned gene libraries (ref. 8 and P.H., G.H. and P.L., unpublished observations) raised the possibility that these fragments also encoded dispersed gene sequences. Therefore, we performed structural and gene mapping experiments on one of the most closely related of these, the 5-kb fragment referred to as $\psi 1$. It was cloned from purified genomic fragments of human lymphocyte DNA using the human λ constant-region probe in conditions of relaxed hybridization stringency (Fig. 1). Hybridization of the cloned, distantly related $\lambda \psi 1$ subfragment back to the genomic DNA exhibits the reciprocal pattern of fragments identified by the active λ gene probe (Fig. 1, lane d); that is, strong hybridization to the 5-kb fragment (itself) and weak hybridization to the active λ genecontaining fragments.

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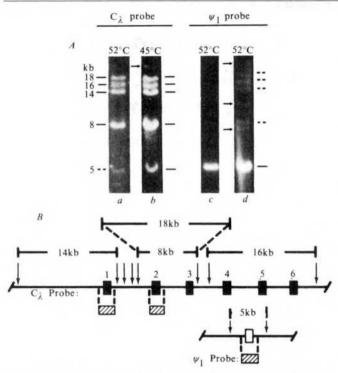


Fig. 1 Identification of cross-hybridizing DNA fragments related to human immunoglobulin A genes. Genomic DNA was extracted from human spleen cells32, digested with EcoRI restriction endonuclease, size-fractionated by agarose gel electrophoresis (10 μ g per lane), transferred to nitrocellu-lose³³, hybridized with ³²P-labelled C λ (a,b) or ψ 1 (d)³⁴, washed in 0.1× SSC, 0.1% SDS at 52 °C (a, d) or 45 °C (b), and visualized by autoradiography. A control lane containing the cloned 5-kb EcoRI fragment is included (c). DNA fragments showing strong hybridization at 52 °C to the $C\lambda$ probe (a) are the 8-, 14-, 16- and 18-kb $C\lambda$ gene-bearing EcoRI fragments. In addition, a 5-kb EcoRI fragment hybridized weakly at 52 °C. When the stringency of the wash conditions was lowered to 45 °C (b), the 5-kb EcoRI fragment hybridization increased. This fragment was cloned and a 750-bp PstI fragment was shown to contain homology to CA. When this 750-bp fragment (see $\psi 1$ probe (B)) was hybridized to the cloned 5-kb EcoRI fragment (c) or human spleen DNA (d), a 5-kb EcoRI fragment hybridized strongly. The 8-, 14-, 16- and 18-kb CA gene-bearing fragments hybridized weakly (d) in these conditions. Four additional bands can be seen hybridizing very weakly and are indicated by arrows (see b and d). The Cλ probe (0.7-kb EcoRI fragment containing coding and flanking sequences of the human A Mcg gene and a 3.4-kb EcoRI-HindIII fragment containing coding and flanking sequences of the \(\lambda \) Kern \(\Oz^+ \) gene) and the \$\psi 1\$ probe (0.75-kb PstI fragment containing \$\psi 1\$ J-C processed gene) are shown in B.

Comparison of the active and pseudogene sequences

The region containing the λ -like sequence was subcloned from the $\lambda \psi 1$ hybrid phage and its nucleotide sequence determined according to the strategy shown in Fig. 2B. Similarly, the sequence of an active λ gene (gene 1, Fig. 1, $Kern^-Oz^+$) was determined for comparison. The two sequences (Fig. 2A) are extensively homologous over the region that corresponds to λ constant region (positions 108–540). Nevertheless, several insertions and deletions alter the $\lambda \psi 1$ sequence so as to create missense runs and termination codons, rendering it a pseudogene.

The points of divergence between the two genes are highly significant. On the 5' side of the sequence, homology ends at the border of the intervening sequence that would have been expected to separate C and J regions in a normal λ gene. A consensus RNA splice site, CCGCAG (PyPyNPyAG), present in the normal gene, is absent from the pseudogene. At the 3' side, homology ends abruptly 26 nucleotides to the 3' side of the putative poly(A) addition signal (AATAAA) that is conserved in both genes. In the pseudogene, the sequence has been replaced at each of these points of divergence by what seems to be evidence of RNA-type processing. The sequence on the 5' side closely resembles a J region complete with reasonable

5' V-J recombination signals spaced the required 12 bases apart 12,13. This homology to an authentic J region can be shown by comparing its nucleotide sequence with that of a known mouse J region (59% homology) and by comparing its translated amino acid sequence with that of a known human λ chain (Fig. 3). The sequence on the 3' side is replaced by a 33-base long poly(A) sequence interspersed with four C and two G residues. The comparable sequence in the active gene is T-rich. Also, a 9-base sequence occurs precisely at the termination of the poly(A) sequence (positions 673-681; Fig. 2A) that is repeated in the 5' portion of the processed gene (positions 15-23; Fig. 2A). This 9-base direct repeat may be the result of an insertion site duplication. In sum, the two sequences diverge from one another at sequences in the pseudogene that resemble those set in place by the two hallmark reactions of RNA processing, the splicing of coding sequences and the addition of poly(A)

Although the pseudogene closely resembles a processed mRNA rather than an intact gene in these important respects, it differs from a normal mRNA sequence in one important way. An active immunoglobulin gene is formed by the joining of a germ-line V region to a germ-line J sequence 12-14. In a few examples thus far studied, the site of transcriptional initiation of these active genes is provided by the incoming V region. Thus, the normal transcriptional initiation site is ~30 nucleotides 5′ from the beginning of the V-region coding sequence 15. The pseudogene sequence noted above completely lacks a joined V-region sequence and indeed retains a reasonable copy of a V-J (DNA) joining signal. Therefore, its similarity to a normal mRNA breaks down in that it lacks the 5′ portion of a normal λ mRNA, the V region, and hence lacks homology to a normal immunoglobulin promoter. We shall return to this problem below.

The processed pseudogene is also dispersed

The initial example of what we refer to as a processed gene, the mouse $\alpha\psi 1$ sequence, resides on mouse chromosome 15, whereas the active mouse α -globin locus is on chromosome 11 (refs 3, 4). Evidently, this processed gene must have been conveyed by some transpositional mechanism from the major globin locus. As gene movement might have a significant role in the formation of processed genes, it was obviously of great importance to determine whether or not the processed λ sequence could be linked to the main λ locus on chromosome 22 (ref. 6). The relationship of these genes was determined in mouse \times human hybrid cell lines that retain different human chromosomes by detecting characteristic restriction endonuclease fragments corresponding to each gene in the various hybrid cell lines 16.

Figure 4 gives an example of such an analysis. Cell line 2 retains the active genes as well as the processed gene, while cell line 1 retains only the active genes. In contrast, cell line 8 retains the pseudogene, but lacks the active genes. As cell lines 1 and 2 retain chromosomes 22 and the cell line 8 does not, these results are consistent with the absence of the processed gene from the normal λ locus on chromosome 22. Similar studies on 24 hybrid cell lines are consistent with this conclusion $(\lambda^+, \psi \lambda^+, \text{ three cell lines}; \lambda^-, \psi \lambda^+, \text{ six cell lines}; \lambda^+, \psi \lambda^-, \text{ three cell lines}; \lambda^-, \psi \lambda^-, 12 cell lines; data not shown), but they do not yet allow us to specify unambiguously the chromosomal location of the processed gene.$

Evidence of RNA-type processing

The spliceless α -globin pseudogene initially suggests that its formation might have been mediated by its processed RNA transcript^{1,2}, and this is supported in several important respects by the discovery of the immunoglobulin processed gene described here. First, the λ -processed gene also bears evidence of two RNA processing events; it has lost its intervening sequences in accordance with the rule of RNA splicing and, like the globin processed gene, its homology with its normal counterpart ends abruptly at a poly(A) addition site. However, unlike the

globin processed gene, its sequence at this point is joined to an extensive poly(A) region (Fig. 2). These features support the notion that processed RNA transcripts were involved in the formation of both genes.

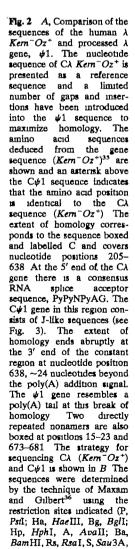
But what of the 5' ends of the two processed genes? Neither of these resembles that of its normal mRNA analogue. The globin sequence homology extends beyond the site of normal transcription initiation and the immunoglobulin sequence clearly has no normal counterpart to the 5' portion of an active λ mRNA. It is therefore clear—if these processed genes were formed from RNA transcripts—that they were aberrantly initiated. (There is some evidence for the formation of such aberrant transcripts in globin and immunoglobulin-producing cells¹⁷⁻¹⁹.) If this is indeed the case, it follows that this 'aberrant' transcript contains genetic information normally excluded from mature mRNAs.

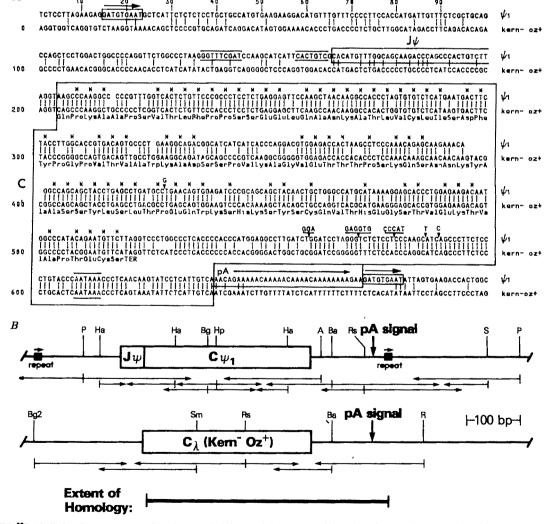
Potential mechanisms for processed gene formation and conveyance

Goff et al.⁵ have noted several useful features of the retroviruses that might account for the conveyance and structure of processed genes. A genomic fragment of DNA, once incorporated into a retrovirus sequence, could be processed so as to lose its intervening sequences during the RNA phase of the viral life cycle. Indeed, there are several examples of incorporate host genes that are represented in spliced form in the retrovirus^{5,20}. However, for such a vector to have incorporated one

of the processed globin or immunoglobulin genes, we would have to postulate a special mechanism to explain how the sequence homology of the processed genes ends at the poly(A) addition site. This feature suggests that the sequence would have to have been taken up as a processed RNA rather than first incorporated, unprocessed, into the retroviral sequence. While this does present a difficulty, gene mapping studies indicate that two retrovirus-like sequences flank the processed α -globin gene, although their configuration does not accommodate a simple model for the role, if any, a retrovirus may have had in their formation²¹.

Two other classes of genomic sequence have also recently been implicated in the formation of processed genes. These are multi-gene families that correspond to the middle repetitive Alu genes²² and the multiple snRNA genes that encode the ubiquitous small nuclear RNAs prevalent in human (and other) . Members of both gene families are transcribed into small RNAs of unknown function²⁴⁻²⁷, the Alu sequence apparently in response to RNA polymerase III²⁸, the snRNA sequence in response to RNA polymerase II²⁹. Although there are multiple active copies of both gene sequences, recent structural studies suggest that many members of these repeated families are pseudogenes, that is, genes that differ from the major expressed version of the sequence^{23,24}. In addition, several features of these genes are similar to integrated transposons: they are flanked by short direct repeat sequences reminiscent of target site duplications, and they are dispersed in the sense that they do not occur in large tandem arrays. (A curious 3.6-kb insertion





Bg2, $Bg\Pi$; Sm, SmaI; R, EcoRI. ¹²P-labelled ends are represented by short vertical lines and the extent and direction of sequencing are indicated by arrows. The boxed area of $C\lambda$ (Kem^-Oz^+) indicates the coding region (amino acids 108-215 of λ polypeptide) and 3'-untranslated region of the mRNA. The boxed area of ψ 1 indicates the sequence that corresponds to J- and C-type sequences. Extent of homology between these genes is shown by a bold black line.

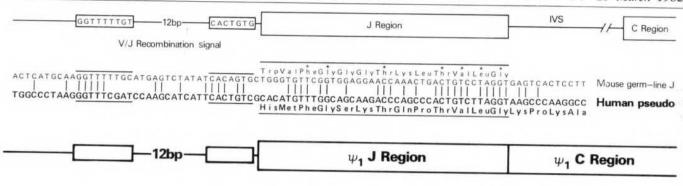


Fig. 3 Comparison of the J-like region of the processed λ gene, $\psi 1$, to a germ-line mouse λ J-region sequence and a typical human J-region amino acid sequence. The nucleotide sequence of $\psi 1$ J region is compared with the nucleotide sequence of a mouse germ-line J region³⁷. The amino acid sequences deduced are shown above and below the nucleotide sequences and an asterisk above the mouse germ-line J amino acid indicates that the amino acid position is identical to the human $\psi 1$. A typical human V-J-C sequence determined at the protein level³⁸ is also shown and an asterisk above the human protein sequence indicates that the amino acid position is identical to the human $\lambda 1$. In addition to the homology seen in the J coding region, the human $\psi 1$ contains remnants of the V-J recombination signal seen in the germ-line sequence of the mouse (indicated by horizontal lines above and below the nucleotide sequence). The homology between the mouse germ-line J and human $\psi 1$ breaks down 3' to the J region, an area where the mouse sequence corresponds to intervening sequence, while the human $\psi 1$ is homologous to CA sequence.

GlySerAspAsnPheValPheGlyThrGlyThrLysValThrValLeuGlyGlnProLysAla Typical human V/J/C

has been found in a *Drosophila* ribosomal gene that is bracketed by 13-base pair (bp) direct repeats³⁰. Careful review of this sequence shows it to end in an A-rich sequence near a poly(A) addition signal.) An additional feature of the two former classes of pseudogene is that their 5' ends, unlike the processed genes we have described, coincide with the normal transcription initiation sites of their RNAs. This fact, and the fact that some, but not all, of these pseudogenes terminate in a poly(A) sequence, have led two groups^{23,24} to propose that they represent a class of transposable element that passes through an RNA intermediate.

Keeping these more detailed proposals in mind, we will consider a minimalist model for the formation of the globin and immunoglobulin processed genes (Fig. 5). First imagine that these processed genes arise from aberrant transcripts that begin at some distance 5' to the genes' normal initiation sites. These transcripts are processed, coding sequences are spliced and poly(A) added. The RNA is converted to DNA and then re-integrated into chromosomal DNA. It is also conceivable that a special sequence has been incorporated into the processed transcript that is necessary for its integration, for example, a retrovirus sequence as suggested by Goff et al.⁵, or an Alu or snRNA element as suggested by Van Arsdell et al.²³ and

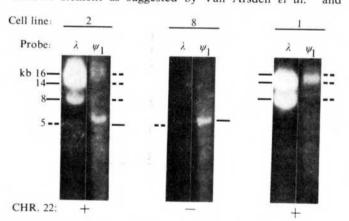


Fig. 4 Discordance of normal and processed λ genes in mouse × human cell hybrids. DNA isolated from somatic hybrid cell lines was digested with EcoRI, fractionated by agarose gel electrophoresis (30 μ g per lane), transferred to nitrocellulose³³, hybridized with a human $C\lambda$ probe or $\psi 1$ probe (see Fig. 1)³⁴ and visualized by autoradiography. Human chromosome 22 carries the active human λ gene locus and is present in cell lines 2 and 1 (ref. 6). The 5-kb EcoRI fragment containing $\psi 1$ gene segregates discordantly with chromosome 22 because it appears in cell line 8 (a cell line that does not contain chromosome 22) and does not appear in cell line 1 (a cell line that does contain chromosome 22).

Jagadeeswaran²², or some other, undiscovered, sequence that shares the mobility properties tentatively ascribed to these elements. Such mobility elements would not normally be transcribed into mRNAs because they lie beyond the usual transcription boundaries of an active gene. Remember that these hypothetical events must have occurred in a germ cell where transcription boundaries and gene activities may differ dramatically from those found in terminally differentiated cells.

If integration of a processed gene does not require a specific integration element and is a fundamental property of any RNA or its cDNA cognate, processed genes might arise from any transcribed sequence. If so, we would expect eventually to find a processed gene that corresponds precisely at both 5' and 3' ends to a normal mRNA. On the other hand, if a mobility element is required, we might expect to find copies of the mobility element still encoded near the parental locus and homologous to a sequence that has been transposed with the processed gene. We might also expect this element to be present near other processed genes. Finally, since we might expect that such sequences would remain a source of these processed genes for a long period of evolutionary time, we might expect to find further examples of processed genes that originate from the

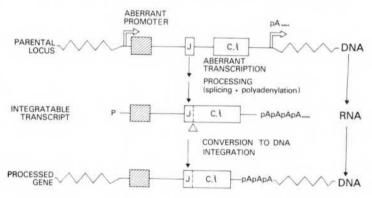


Fig. 5 A minimal model for the formation of the processed Cλ gene. The top line represents the germ-line configuration of the putative ancestral Cλ gene, showing (in boxes) the J and C coding regions as well as the site of a proposed aberrant promoter (arrow) and of poly(A) addition (arrow). The hatched box represents a hypothetical sequence that might render a transcript of this region integratable, a detail that is not required if we assume that any RNA sequence (or its cDNA cognate) can ultimately be integrated into chromosome DNA. The proposed scheme simply calls for an aberrant transcript of this sequence to arise in a germ cell, be processed as an RNA (spliced and polyadenylated), then be converted into DNA for subsequent (or simultaneous) incorporation into a new chromosomal location. Note that information flows from DNA to RNA back to DNA.

affected member of the active gene family. We already know that there are additional, unlinked copies of both genes that are processed gene candidates. It is therefore possible that processed genes represent a major class of genomic element and, while several mechanisms are available for their conveyance, their structures strongly support the notion that RNA transcripts have been involved in their formation. Consistent with this notion, N. Cowan and colleagues (personal communication) have discovered an interesting human α -tubulin-like

Received 11 December 1981, accepted 12 February 1982

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pseudogene that has lost its intervening sequences and ends in a poly(A) tail. We expect many such processed genes will be discovered as other multi-gene families are examined in greater detail. Thus, the notion that information can flow from DNA to RNA back to DNA, long established in the case of the retroviruses³¹, seems to involve non-viral genes as well.

We thank Ms Terri Broderick for assistance in the preparation of this manuscript, Aya Leder for helpful discussions and Debra Keithley for technical assistance.

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Immunoglobulin heavy-chain expression and class switching in a murine leukaemia cell line

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A cell line that switches from μ to $\gamma 2b$ synthesis during growth in culture uses the same V_H region for both heavy chains but retains two copies of the C_{μ} gene. This suggests that the μ to $\gamma 2b$ class switch can occur, at least in part, by an RNA processing mechanism. Regulatory variants of this cell line lose constitutive μ -chain synthesis but simultaneously acquire lipopolysaccharide(LPS)-inducible synthesis of that chain. This co-variation is allele-specific and is correlated to a large deletion of DNA in the J_H - C_μ intron.

INFECTION of murine bone marrow or fetal liver cells with Abelson murine leukaemia virus (A-MuLV) transforms a small fraction of the cells into clonal, continuous cell lines¹. Various properties of these lines indicate that they are generated from immature cells of the B-lymphoid (immunoglobulin-producing) cell lineage, the majority being related to the most immature cell known to occur in this pathway, the 'pre-B' cell²⁻⁴. Surveys of the structure and expression of immunoglobulin genes in large numbers of such lines have proved extremely useful for defining the pre-B stage of differentiation at a molecular level. In addition, these studies have defined specific subclasses of A-MuLV transformants which exhibit individual aspects of the immunodifferentiation programme. One such cell line is 18-8, and its clonal derivative 18-81, which usually produces μ chains in the absence of detectable light chains² but from which certain clonal isolates also produce κ chains after stimulation with bacterial lipopolysaccharide (LPS). The latter phenomenon is probably due to productive κ -gene rearrangement during growth of the 18-8 line in culture^{5,6}. Synthesis of γ -related

chains has also been detected in populations of these cells² and in subclones of these lines. The work of Burrows et al. 7. together with our preliminary studies⁵, suggested that during growth in culture, the 18-81 cell line may undergo heavy-chain class switching; this is the process by which a clone of Blymphoid cells first expresses a single heavy-chain variable (V) region in association with μ heavy chains and subsequently with another heavy-chain class such as α or γ (ref. 8). The process often appears to be effected by a recombination/deletion mechanism. The order of the CH genes has been determined as $5'-(V_HJ_H)-C_{\mu}-C_{\delta}-C_{\gamma3}-C_{\gamma1}-C_{\gamma2b}-C_{\gamma2a}-C_{\epsilon}-C_{\alpha}-3'$ (ref. 9) so that, for example, expression of $\gamma 2b$ protein in a myeloma would result from juxtaposition of the C_{v2b} gene with the expressed V_HDJ_H complex by deletion of the intervening DNA sequence, including the C_{μ} , C_{δ} , $C_{\gamma 3}$, and $C_{\gamma 1}$ genes^{10,11}. Most of the evidence for the deletional model of heavy-chain class switching has come from comparative studies of heavy-chain gene structure in independently derived immunoglobulin-secreting myelomas⁹⁻¹⁶. Clearly, cloned cell lines such as 18-8, which undergo class-switching in culture, could be useful for elucidating this process. .

In addition, certain properties of 18-8 and its sublines suggest that these cells may be useful for generating variants having

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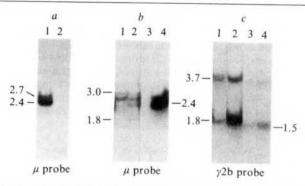


Fig. 1 Detection of μ and γ 2b RNA sequences in various 18-81 isolates. The derivation of the 18-8 line and its clonal derivative 18-81 has been described previously2. Subclones of 18-81 were derived by dilution and plating in microtitre wells at <1 cell per well. For LPS treatment, cells were plated at 4×10^5 cells ml⁻¹ and grown in the presence of $100 \,\mu g \, ml^{-1}$ Salmonella typhimurium LPS (Difco) for $48 \, h^{17,34}$. Parallel control cultures were evaluated in each experiment. To examine the RNA in the various 18-81 cultures, ~10 µg of total poly(A)-containing RNA prepared from the indicated sources was fractionated by electrophoresis through methylmercury hydroxide agarose gels, transferred to diazotized paper and hybridized with $\sim 10^7$ c.p.m. of a nick-translated cloned DNA probe for either μ sequences $(pAB\mu - 1)$ or $\gamma 2b$ sequences $(pAB\gamma 2b-1)$ (specific activity between 10^8 and 10^9 c.p.m. μg The pAB μ - 1 plasmid contains a 770-base pair (bp) μ cDNA and the pAB γ 2b-1 a 1,200-bp γ 2b cDNA insert⁴⁰. a, μ probe, RNA from: lane 1, young 18-81 culture; lane 2, 18-81A. b, μ probe, RNA from: lanes 1 and 2, 81A-2 cells; lanes 3 and 4, 81A-20 cells. Cells were either untreated (lanes 1 and 3) or treated with LPS (lanes 2 and 4). c, γ 2b probe: lanes as indicated for b.

altered regulation of immunoglobulin synthesis. During propagation of the 18-81 line in culture, its relatively high level of μ -chain synthesis (0.1% of soluble protein) decreases; after 9–12 months of culture it makes <10% of the μ chain found in the parent population 17 . The synthesis of μ chains in long-term cultures of 18-81 can be increased to a level approaching that of fresh cultures by treatment of the cells with bacterial LPS 17 . This suggests that growth of 18-8 cells in culture may select for variants having stable changes in the regulation of immunoglobulin heavy-chain gene expression. Such variants would be invaluable for studying the molecular basis of the various transitions in the level of heavy-chain gene expression that occur during B-cell differentiation.

Here we characterize cloned variants of the 18-8 line which exhibit one or both of the properties described above. The studies indicate that in these variants, heavy-chain class switching may occur without deletion of the C_μ gene and that the genetic alteration leading to decreased heavy-chain expression (and apparently the simultaneous acquisition of LPS inducibility of expression) is linked to DNA alterations near the functionally expressed $V_{\rm H}$ allele.

Loss of μ production during culture correlates with decreased levels of μ mRNA

To characterize the events involved in the decrease of μ protein synthesis in derivatives of cell line 18-8, we first analysed the relative level of μ -related RNA sequences in a line cloned soon after the establishment of 18-8 (called 18-81) and in the same line after 9 months of continuous passage (called 18-81A). Line 18-81 produced ~0.1% of its protein as μ chains; 18-81A had no detectable μ chains. For these analyses, total poly(A)-containing RNA was prepared, fractionated, transferred to diazotized paper and assayed for hybridization to 32 P-labelled μ -specific cloned DNA probes as described previously 18 . Hybridization with a probe specific for C_{μ} revealed a prominent 2.4-kilobase (kb) μ RNA, as well as a minor 2.7-kb species in the young culture (Fig. 1a, lane 1) but no detectable μ RNA in the long-term culture (Fig. 1a, lane 2). Hybridization with

sub-probes specific for $\mu_{\rm m}$ or $\mu_{\rm s}$ domains demonstrated that the 2.4- and 2.7-kb μ RNAs in the parent populations corresponded respectively to $\mu_{\rm s}$ and $\mu_{\rm m}$ RNA (ref. 18). Therefore, the decrease in μ protein production that occurred when the 18-81 line was grown in culture correlated with a reduction in μ mRNA accumulation.

Subclones of 18-81A produce either LPS-induced μ or γ 2b protein and mRNA

After ~9 months in culture the 18-81A line was subcloned to produce the 81A series of cell lines. Individual 81A lines were then examined for heavy-chain production by metabolic labelling and specific immunoprecipitation. Consistent with the behaviour of the parental line, of 40 81A lines examined, none constitutively synthesized significant levels of µ-related proteins. However, all but one of the lines synthesized μ protein after treatment with LPS; the behaviour of a representative line, 81A-20, is shown in Fig. 2, lanes 1 and 2. One line, 81A-2, produced no μ with or without LPS treatment but produced detectable levels of a y chain in the absence of LPS treatment (Fig. 2, lane 3) and greatly increased amounts after LPS treatment (Fig. 2, lane 4). Analyses using sera specific for various γ classes indicated that the γ produced by 81A-2 was γ 2b (not shown). This line also produced low levels of a μ -sized chain that was precipitated in our assay conditions (Fig. 2, lane 4), but which does not appear to represent μ protein because: (1) the precipitation of this protein occurred at variable levels; (2) it was observed with Staphylococcus aureus treatment alone; (3) it was not observed with double antibody precipitation; and (4) its precipitation was not competed with authentic μ protein (data not shown).

To ascertain that the γ -producing 81A-2 clone, the μ -producing 81A-20 clone and the 18-81 line were all sibling clones, we examined their integrated A-MuLV proviruses⁵. All the clones had identically integrated proviruses⁵, and must therefore have been descendents of the same, initially transformed cell because individual A-MuLV transformants have distinguishable proviruses¹⁹.

Analyses of the RNAs produced by the 81A lines yielded results parallel to those obtained by protein analyses. The μ -inducible, 81A-20 line contained a barely detectable level

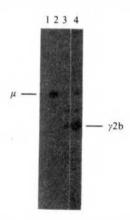


Fig. 2 LPS enhancement of μ and γ 2b production in various 18-81 isolates. Cell lines 81A-20 and 81A-2 were treated with LPS as described in Fig. 1 legend, then metabolically labelled with 35 S-methionine (80 μ Ci ml $^{-1}$) for 60 min as previously described 2,18,41,42 . Control cultures which did not receive LPS were incubated and labelled in parallel. Extracts were then prepared and incorporation into μ - or γ -related polypeptides determined by precipitation with rabbit anti-mouse immunoglobulin and SDS-polyacrylamide gel electrophoresis of the resulting precipitates as previously described 2,18,41,23 . Lane 1, 81A-20, no treatment; lane 2, 81A-20, LPS; lane 3, 81A-2, no treatment; lane 4, 81A-2, LPS. Identity of μ and γ chains was confirmed by specific immunoprecipitation and competition assays as previously described 18 .

of 2.4-kb μ_s mRNA which was greatly augmented by LPS treatment (Fig. 1b, lanes 3 and 4). A 1.5-kb γ -related RNA, the level of which was not significantly affected by LPS treatment, was also evident in this line when the same RNA was assayed using a γ 2b probe (Fig. 1c, lanes 3 and 4). This RNA species, however, does not appear to contain a V_H sequence (see Fig. 4) and is probably related to the 'sterile μ ' RNA transcripts described below.

The y2b-inducible 81A-2 line produced a diffuse pattern of μ RNA species ranging in size from 3.0 to 1.8 kb and the abundance of these species was not affected by LPS (Fig. 1b, lanes 1 and 2). These 'sterile μ ' RNA sequences²⁰, which are probably related to those described by others21, are found in many A-MuLV-transformed cell lines and do not encode µ chains²⁰. The 81A-2 line also contained 1.8- and 3.7-kb species of y-related RNA (Fig. 1c, lane 1); the level of the 1.8-kb species was greatly augmented by LPS treatment (Fig. 1c, lane 2). Characterization of these RNAs with DNA probes specific for the various v subclasses confirmed their identity as mRNAs for the membrane-bound (3.7 kb) and secreted (1.8 kb) forms of γ 2b protein^{22,23}. Thus, as observed by analyses of both protein and RNA, clone 81A-2 has apparently stably shifted from μ to $\gamma 2b$ production in culture. In addition, like μ expression in the parental line or sibling clones, γ 2b expression is low in 81A-2 but inducible to high levels by treatment of the cells with LPS.

Heavy-chain gene rearrangements in the 18-8 series

To examine whether the switch to γ 2b production in 81A-2 was paralleled by changes in the cell DNA, we performed a

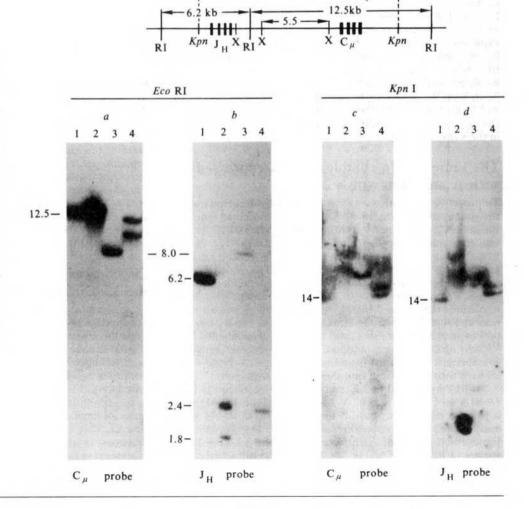
restriction endonuclease analysis of the C_μ and J_H regions in the DNA of 81A-20 and 81A-2. In the germ line of the BALB/c mouse, all the C_μ exons occur on a single EcoRI fragment of 12.5 kb, called here the RI- C_μ fragment, and the J_H segments are located on a contiguous 6.2-kb EcoRI fragment, called here the RI- J_H fragment (Fig. 3). Any rearrangement in which a V_H or D_H region is abutted to a J_H should abolish the germ-line RI- J_H fragment and generate a new fragment containing the DNA sequence lying between the active J_H and the nearest 3' EcoRI site.

To assay the DNA segments in various cell lines, DNA was prepared, digested with EcoRI and assayed for hybridization to ^{32}P -labelled DNA probes representing either the RI-J_H fragment⁵ or the C_{μ} region 18 . As observed with embryo DNA (Fig. 3a, lane 1), a major, 12.5-kb EcoRI fragment in the DNA of the 18-8 parental line hybridized to the C_{μ} probe (Fig. 3a, lane 2). When assayed with the RI-J_H probe, however, two fragments of \sim 2.4 and 1.8 kb were observed in the parental 18-8 line (Fig. 3b, lane 2), neither of which corresponded to the unrearranged 6.2-kb fragment observed in DNA from mouse embryo (Fig. 3b, lane 1). This result indicates that both $J_{\rm H}$ alleles had undergone rearrangement in the 18-8 line and was confirmed by demonstrating that the 1.6-kb EcoRI fragment directly 5' to the RI-J_H fragment in embryonic DNA was absent from the genome of 18-8 and its daughter lines⁴.

In the DNA of the 18-81A line, only a single EcoRI fragment of 8.0 kb hybridized to the C_{μ} probe (Fig. 3a, lane3). In addition, this cell population contained only a single observable RI-J_H fragment which was identical in size (8.0 kb) to that containing the C_{μ} gene (Fig. 3b, lane 3); this was confirmed by eluting the RI-J_H probe and rehybridizing with the C_{μ} probe (data not shown). These results imply that one J_{H} - C_{μ} allele had been lost

14 kb

Fig. 3 Analysis of heavy-chain gene rearrangements in various 18-8 isolates. Upper part, restriction map of J_H-C_{\(\mu\)} region. Relevant EcoRI (RI), KpnI (Kpn) and XbaI (X) sites are indicated. Lower part, approximately 10 µg of cel-DNA indicated from sources were digested with EcoRI (panels a, b) or KpnI (panels c, d) and assayed by DNA blotting procedures for hybridization to the 32P-labelled (final specific activity = 10^8 - 10^9 c.p.m. μ g pAB μ – 1, C_{μ} probe (panels a, c) or the pRI-J_H, J_H probe (panels b, d) as described elsewhere4. Plasmid pAB μ - 1 is described in Fig. 1 legend; pRI-J_H has a 6.2-kb insert which corresponds to the embryonic EcoRI fragment containing the JH segments (see map above). Lane 1, BALB/c embryo DNA; lane 2, 18-8 DNA; lane 3, 18-81A DNA; lane 4, 81A-2 DNA.



in the 18-81A population whereas the other had undergone a deletion of \sim 5-6 kb spanning the EcoRI site between RI-J_H and RI-C_m.

The location of the deletion in the single J_H - C_μ region in line 18-81A was also confirmed by hybridization with sub-probes specific for this intron region (see below) and by cleavage analyses with other enzymes. An identical 8.0-kb fragment hybridizing to both the RI- C_μ and RI- J_H probes was also observed in the DNA of 81A-20, the μ -inducible clone (data not shown), implying that the heavy-chain allele containing the large deletion—the only one present—is the one expressed in this and therefore the other 18-8 related lines. In addition, a loss of one C_μ allele and a deletion in the other occurred when a separate population of 18-81 was maintained in culture until μ -gene expression substantially decreased (data not shown).

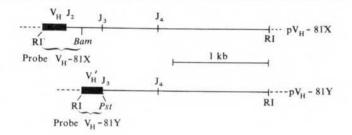
Analysis of the RI-J_H region in the DNA of the $\gamma 2$ b-producing derivative, 81A-2, indicated the presence of RI-J_H regions originating from both chromosomes and showed no evidence of rearrangement with respect to the configuration found in the 18-8 ancestral line (that is, 2.4- and 1.8-kb fragments; Fig. 3b, lane 4). In addition, two RI-C_{μ} fragments of 11.0 and 9.5 kb were found in the DNA of this line (Fig. 3a, lane 4). This result suggested that the shift to $\gamma 2$ b production in 81A-2 may have occurred without deletion of a C_{μ} gene.

To examine further whether 81A-2 retained both C_µ alleles, we analysed the J_{H} - and C_{μ} -containing fragments generated when the DNA of this line was digested with KpnI. Figure 3 shows that KpnI cuts 3' to the C, exons and 5' to the JH segments, thus generating a single 14-kb embryonic DNA fragment containing both these regions. Rearranged genes, in which the C., regions are still associated with the JH region, should result in identical patterns of hybridization when KpnI-digested DNA is assayed with either RI-J_H or C_µ probes. Juxtaposition of the JH region to a y2b allele, however, might be expected to produce a unique KpnI fragment when assayed with RI-JH. As predicted, C_μ and RI-J_H probes showed identical patterns of hybridization to a 14-kb KpnI fragment of embryonic DNA (Fig. 3c, d, lanes 1) (the C_u-containing fragment was more distinct in other experiments). In DNA from line 18-8, both probes hybridized to 18.5- and 17-kb fragments (Fig. 3c, d, lanes 2). In DNA from 81A-20, the probes located only a single 17-kb fragment, consistent with the presence of only one JH-C region (Fig. 3c, d, lanes 3). In DNA from line 81A-2, two fragments of 16 and 15 kb hybridized to both probes (Fig. 3c, d, lanes 4), suggesting that both J_H alleles were still associated with C_µ genes in the y2b-producing line.

The same V_H - J_H complex is first expressed with μ and then with $\gamma 2b$

To prove rigorously that a switch had actually occurred during the propagation of the 18-81 cell line, we examined whether the same $V_{\rm H}$ gene expressed with C_{μ} in the parental 18-81 line was subsequently expressed in association with $C_{\nu 2b}$ in the 81A-2 subclone. We molecularly cloned both RI-J_H fragments from 81A-2 into λ phage Charon 16A and demonstrated by nucleic acid sequencing that they contained independently rearranged $V_{\rm H}$ genes rearranged to $J_{\rm H}2$ ($V_{\rm H}$ -81X) and $J_{\rm H}3$ ($V_{\rm H}$ -81Y) (structures summarized in Fig. 4; data to be presented elsewhere). From these two clones we prepared the two $V_{\rm H}$ -specific probes as indicated in Fig. 4, and these were then labelled with ^{32}P by nick-translation and used to probe total poly(A)-containing RNA isolated from lines 18-81A, 81A-2 and 81A-20 both with and without (not shown) LPS treatment.

 V_H -81Y hybridized to 2.7- and 2.4-kb RNA species in both 18-81A (not shown) and 81A-20 RNA (Fig. 4a, lane 1) with a pattern identical to that obtained when these RNAs were assayed with a C_μ probe (Fig. 1a, lane 1; Fig. 1b, lane 4). Furthermore, this probe hybridized to 1.8- and 3.7-kb γ RNA species in 81A-2 RNA (Fig. 4a, lane 2), a result identical to that obtained when this RNA sample was assayed with a γ 2b probe (Fig. 1c, lane 2). The V_H -81X probe did not hybridize



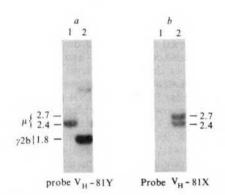


Fig. 4 Upper part, partial restriction map of clones pVH-81X and pV_H-81Y. The plasmids pV_H-81X and pV_H-81Y have, respectively, 2.4- and 1.8-kb inserts containing the rearranged V_HJ_H complexes from 81A-2 as indicated in the map. The plasmids were derived by subcloning the inserts from EcoRI-digested Charon 16A genomic clones into the EcoRI site of pBR322. All procedures conformed to the NIH guidelines for recombinant DNA research. The probes V_H-81X and V_H-81Y were prepared by dissecting the indicated restriction fragments from each clone and then purifying these by agarose gel electrophoresis and labelling with 32P by nick-translation as previously described 18. Lower part, expression of rearranged VH genes in 18-81 clones. Approximately 10 µg of poly(A)-containing RNA from 81A-20 (lane 1) and 81A-2 (lane 2) were fractionated by methylmercury hydroxide agarose gel electrophoresis, transferred to diazotized paper and assayed for hybridization to probe V_H-81Y (a) or V_H-81X (b).

to any RNA in 81A-20 cells (Fig. 4b, lane 1), consistent with the loss of this allele from the cell line (see above). This probe did, however, hybridize to a 2.7- and 2.4-kb $\mu_{\rm m}$ - and $\mu_{\rm s}$ -sized RNA doublet in 81A-2 RNA (Fig. 4b, lane 2) but these sequences apparently result from a non-productive rearrangement at the unexpressed allele and do not encode normal μ proteins (see ref. 20). These results confirm that the 81A-2 clone has undergone a class switch from μ to $\gamma 2$ b production using the same rearranged $V_{\rm H}$ -J $_{\rm H}$ region to make both types of RNA. Furthermore, in all these lines, only the RNA sequences hybridizing to the $V_{\rm H}$ -81Y probe showed a significant response to LPS treatment, suggesting that LPS sensitivity was a property of the expressed allele.

Derivatives of 18-8 accumulate deletions in the J_H-C_μ intron during growth in culture

To analyse further the deletions which occurred near the C_{μ} gene in the 18-8 cell lines, we prepared a 32 P-labelled probe from the major XbaI fragment lying within the J_H - C_{μ} intron (Fig. 3) and used it to analyse the size of homologous fragments in XbaI-digested DNA from the various lines. As expected, this probe labelled a prominent 5.5-kb XbaI fragment in embryonic DNA (Fig. 5, lane 1). In DNA from the 18-8 line, the intron probe labelled both the 5.5-kb fragment as well as a series of smaller fragments ranging in size to <3 kb (Fig. 5, lane 2). This suggested that the 18-8 line was accumulating deletions in this region during growth in culture. Similar analyses revealed no size heterogeneity of restriction fragments representing sequences lying 5' or 3' to those represented by the intron probe (data not shown), supporting the proposal that

sequences within the region covered by the probe have a predisposition for deletion, as suggested by others2

Additional support for the suggestion that 18-8 accumulated J_H-C₄ deletions during culture was provided by the observation that DNA from the 81A-20 and 81A-2 clones did not contain XbaI fragments of embryonic size that hybridized with the intron probe (Fig. 5, lanes 3 and 4). Although both Cu alleles appeared to be present in the 81A-2 line, the XbaI-digested DNA from this line contained only one obvious fragment of ~2.3 kb which hybridized to the intron probe (Fig. 5, lane 3). Analyses using other enzymes and probes indicated that the lack of a second hybridizing fragment probably resulted from a deletion in the second allele which removed most of the sequence homologous to the probe (data not shown), a result consistent with the small size (9.0 kb) of one of the Cucontaining EcoRI fragments in this line (Fig. 3a, lane 4). Similarly, from other experiments, the large size (5 kb) and weak hybridization of the single hybridizing XbaI fragment in 81A-20 DNA (Fig. 5, lane 4) seems to have resulted from a large deletion removing the first two XbaI sites directly 3' to the JH segments (Fig. 3), the 5' XbaI site now being provided by sequence associated with the rearranged VH gene.

Mechanism of switching

Previous evidence has suggested that cells producing μ chains can switch to production of other heavy-chain isotypes^{8,25,3} but only in the case of cells that have activated δ synthesis has evidence been provided that the same VH region is expressed first with μ and then with the derivative heavy chain²⁷. The present report shows that an A-MuLV transformant which has switched from μ to γ 2b synthesis, uses for its γ 2b protein what appears to be the same VH region as used by the parental line for its μ protein. The apparent identity of the V_H regions used

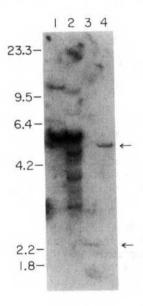


Fig. 5 Deletions within the J_H - C_μ intron. The J_H - C_μ intron probe was prepared by dissecting the 3.5-kb XbaI fragment corresponding to the major 5.5-kb XbaI fragment in the JH-C, intron (see Fig. 3) from a genomic clone (pRI-C_µ) which has a 10.5-kb insert containing the C_µ gene⁴. The insert of this plasmid corresponds to the embryonic 12.5-kb EcoRI fragment which contains the C₄ exons (see Fig. 3) but during cloning has undergone a ~2-kb deletion several kb 5' to the first C_{\(\mu\)} exon. The size of the excised XbaI fragment (3.5 kb) was a result of this deletion. Approximately 10 µg of DNA from the indicated sources were digested with XbaI, fractionated by electrophoresis through 1% agarose gels, transferred to nitrocellulose paper and assayed for hybridization to the J_H – C_μ intron probe labelled with ^{32}P by nick-translation. DNA from: lane 1, BALB/c embryo DNA; lane 2, 18-8; lane 3, 81A-2; lane 4, 81A-20.

in the μ and γ 2b proteins is indicated by the following: the VH genes joined to the JH regions on the two chromosomes in 18-8 cells and its derivatives have been molecularly cloned and are denoted V_H -81X and V_H -81Y; a μ -producing 18-8 derivative has lost one allele but retained V_H-81Y in its μ mRNA, identifying this as the V_H that encodes the μ protein; the $\gamma 2b$ mRNA in the γ 2b-producing derivative hybridized to V_H-81Y but not V_H-81X, identifying V_H-81Y as the V_H region that encodes the γ 2b protein and showing that the μ and γ 2b proteins share VH regions. Actually, the identity of VH regions on the two mRNAs was established mainly by cross-hybridization, but based on the lineage relationships of these lines, the possibility that the cross-hybridizing V_H regions are not identical is very low.

The mechanism of heavy-chain switching has previously been inferred from observations on the configuration of the heavychain genes in myeloma cells, tumour cells that represent terminally differentiated cells of the B-lymphoid lineage. These studies have indicated that class switching occurs by a deletional mechanism9-16, and this process is apparently mediated by switch recombination sites, a set of which lies 5' to each of the C_H genes^{15,16,28,29}. Thus we expected that the switch of 18-81 cells from μ synthesis to γ 2b synthesis would be accompanied by a deletion of at least one copy of the C, gene segment in these cells. However, the y-producing 81-A2 line described here retains two copies of the C_{μ} gene, each of which still appears to be associated with the V_H complex (Fig. 3). Because digests using other enzymes suggest the possibility of an alteration within or near the C_µ gene on one of the alleles (F.W.A. and D.B., unpublished results), additional molecular cloning analyses will be necessary to confirm the linkage. However, analysis of the γ 2b gene in these lines using a series of different restriction endonucleases revealed no obvious alterations near either the y2b gene or its flanking sequences (data not shown). In addition, there are examples of at least two other A-MuLVtransformed lines that apparently have undergone a switch to γ production without loss of a C_{μ} gene (see ref. 20). The data available imply that the switch to $\gamma 2b$ production in A-MuLV transformants results from transcription through the C, gene, with deletion of the C_µ and other intervening sequences occurring by a post-transcriptional processing mechanism. Such a mechanism would be analogous to that proposed as mediating the transition from μ_m to μ_s RNA production 18,30,31 and the simultaneous expression of μ and δ genes by the same cell^{27,32,33}, and predicts either a very long (>50 kb) primary transcript or a rearrangement placing the y2b gene closer to μ , perhaps in the place of δ . Whatever the mechanism, this line should provide an excellent model system for studying various aspects of the class-switching process.

It has been suggested that the occurrence of class switching in 18-81 cells demonstrates that pre-B lymphocytes can carry out the class switch event7. Although it is true that most A-MuLV-transformed cells appear closely related to pre-B lymphocytes, 18-81 may be an exception; as opposed to most other A-MuLV transformants, it gives rise to subclones having rearranged κ light-chain genes^{4,5} some of which produce κ protein^{6,34}, it makes predominantly the secreted form of μ mRNA (Fig. 1), and it probably produces a low amount of J chain (M. Koshland, personal communication). These characteristics suggest that 18-81 is a cell line having a propensity to mature beyond the pre-B-lymphoid cell stage. Thus, the relatively high frequency of γ-producing cells generated by this line may be a consequence of its maturational potential as opposed to an event of the pre-B lymphocyte.

Class switching in cultured cells

Other laboratories have been able to detect among cultured myeloma and hybridoma cells, variants that express a new heavy-chain isotype³⁵⁻³⁷. In these experiments, powerful mutagenic and selective methods were used to recover the very rare class-switch variants which appear in non-mutagenized

cells at a frequency of <10⁻⁶ per cell per generation^{36,37}. Our analyses, together with those of Burrows et al.⁷, suggest that μ to $\sqrt{2}$ b class switching is continually occurring in the 18-8 cell line and in some isolates may occur at a frequency as high as 10⁻² per cell per generation.

A second difference between the selected myeloma and hybridoma variants and A-MuLV transformants is that the 81A-2 line has switched from μ to γ 2b expression, skipping the intervening $\gamma 3$ and $\gamma 1$ C_H regions, whereas the selected variants generally switch to a neighbouring C_H gene³⁵⁻³⁷. The switch from μ to a different heavy-chain isotype is thought to occur normally during B-cell differentiation. Switching from one γ subclass to a different subclass—as occurs in the myeloma variants—has not been reported for normal B cells and the significance of this type of low-frequency switching in permanent cell lines is not clear. A μ to δ class-switch variant has been described³⁷ and probably results from deletion of the C_n gene²⁷.

Regulation of μ expression

The high level of μ -specific protein and RNA expressed by the 18-8 and 18-81 A-MuLV-transformed cell lines (0.1% of soluble protein; see ref. 2) is relatively unique among μ -positive, light chain-negative A-MuLV-transformants, which generally produce ~10-fold lower levels. Previous studies have demonstrated that the production of high levels of heavy chains has an adverse effect on the growth of plasmacytoma lines that have lost light-chain gene expression³⁸. In view of this result, the decline of μ production in long-term cultures of the μ -only 18-8 lines most probably results from the selective outgrowth of cells in which μ production has decreased, while the relative stability of μ levels in many μ -only A-MuLV-transformed lines (N.R. and F.W.A., unpublished results) may reflect the low levels of the protein produced initially.

The decreased μ production in various 18-81 isolates is usually inducible to the original high levels on treatment of the cultures with LPS. We have as yet little information on the genetic basis of the decreased expression or induction phenomena. However, note that in the 81A-2 clone that has switched to γ 2b production, γ 2b protein and RNA sequences show an extent of LPS induction similar to that observed for μ sequences in the parental line whereas the defective μ mRNA sequences produced from the second allele show little or no induction (Fig. 1). This result suggests that the structure mediating the induction is associated with the expressed V_H gene or its flanking sequences and is presumably an element of DNA structure. In this regard it is significant that in the two low-level μ producers that are LPS-inducible, only a single copy of the μ gene was apparent, and this had undergone an extensive deletion in the intron between J_H and C_u. An intriguing possibility is that a sequence in the deleted region has a positive involvement with μ production and that loss of that sequence can be overcome by LPS treatment. It should be noted, however, that deletions within this region of up to 2.8 kb are compatible with high-level μ production in myelomas³⁹.

The deletions that occur within the J_H-C_{\(\mu\)} intron during propagation of the 18-8 line may be related to strain-associated length variations in that region²⁴ as well as to deletions within that region which are observed in myelomas and which occur

when C_{μ} genes are cloned and propagated in *Escherichia coli*^{15,16,24,28}. The latter two phenomena have been ascribed to a propensity of the S_u region—which lies ~2 kb 5' to the C_u gene—to promote deletions 15,16,24,28. Although many of the deletions within this region observed in the 18-8 populations may be analogous in size to those described for myelomas or molecular clones (~2 kb), the deletions observed in some of the 81A clones are much larger (>5 kb). However, these deletions do appear to have their 3' termini near the S_u region. Whether or not all these deletions are also mediated by the S_u region remains to be determined. However, an intriguing possibility is that the high rate of deletion within this region is related to the propensity of the 18-8 line to undergo class switching. Most A-MuLV-transformed lines have deletions within this region (not shown) and the only other line examined in detail, 300-18, also accumulated these deletions during the growth in culture (F.W.A. and D.B., unpublished observations). However, of five permanent T-cell lines examined, all of which had been in long-term culture, none showed evidence of deletions within this region, suggesting that the activity responsible may be B-cell specific.

Conclusion

These data emphasize the variety of phenotypes to be found among the many cell lines transformed by A-MuLV. In this case, subclones from a single transformant, 18-81, have shown a switch from μ to γ 2b synthesis and a progressive loss of heavy-chain gene expression. Analysis of both events has uncovered aspects that may be important to the general understanding of immunodifferentiation.

The switch from synthesis of μ to synthesis of γ 2b was accomplished without the expected loss of a C, gene, which suggests that class switching beyond the δ gene may be, at least partly, a transcriptionally controlled event in contrast to previous evidence for a deletional mechanism⁹⁻¹⁶. Deletion certainly occurs, but it may be secondary to a transcriptional (or RNA processing) switch. The progressive loss of heavy-chain synthesis has two interesting aspects: it can be reversed by LPS treatment of cells and can be correlated with deletion in the J_H-C_u intron. Although the causative relationships have not been proved, the results suggest that transcription of heavychain genes (both μ and γ 2b) or the processing of initial transcripts may be regulated by DNA sequences in the J_H-C_u intron. Both the amount of mRNA produced and the control of mRNA levels by LPS were correlated to the deletion; if these measurements reflect transcriptional activity, immunoglobulin gene expression may be regulated by DNA elements in the J_H-C_u intron rather than any upstream of the V_HJ_HC_H complex. This is a reasonable assumption because otherwise each V_H gene would be required to carry a complete regulatory region 5' to its structural gene. The way in which elements of the intron may regulate transcription requires further study.

This work was supported by grants from the American Cancer Society (to D.B.) and the NCI (to D.B. and N.R.) and by a contribution from the Whitehead Charitable Foundation. F.W.A. was a Special Fellow of the Leukaemia Society of America. N.R. is the recipient of a Research Career Development Award from the NCI. D.B. is an American Cancer Society Research Professor.

Received 26 October 1981, accepted 21 January 1982

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Speckle observations of the nucleus of NGC1068

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The nuclei of Sevfert galaxies contain very turbulent motion and are often intense sources of radio emission. It has been proposed that very compact supermassive objects could be the source of these energetic phenomena. Speckle observations of the nucleus of NGC1068 have revealed for the first time the presence of such an object with a diameter of ≤2.3 pc but emitting an amount of visible light equivalent to that from 5×10^9 solar masses.

NGC1068 is an Sb spiral galaxy1 with a Seyfert nucleus2 which is at distance³ of 16 Mpc (assuming H_0 = 75 km s⁻¹ Mpc⁻¹). Several observers³⁻⁶ have studied the broad (≈1,450 km s⁻¹) [O III] emission lines from the central (=300 pc diameter) regions. There are at least four distinct clouds with separate velocities within this range and the total kinetic energy³ in this turbulent ionized gas is $\approx 9 \times 10^{54}$ erg. Bertola⁶ has detected a stellar-like inner nucleus with a diameter of $\leq 160 \,\mathrm{pc} \,(\leq 2 \,\mathrm{arc}\,\mathrm{s})$ which emits 9% ($\simeq 12 M_{\rm v}$) of the total visible continuum of the galaxy. The variability of this optical emission has suggested that this may originate within a volume as small as a few light months.

High-resolution (=1 arc s) radio observations of the nuclear regions of NGC1068 by the Jodrell Bank MERLIN array8 and VLA9 reveal that an intense source of non-thermal radio emission of \approx 77 pc diameter (\approx 1 arcs) is coincident with this central object. This is of size comparable with the upper limit of the 10 µm IR structure¹⁰, whereas radio VLBI measurements^{11,12} have, so far, failed to detect emission on an angular scale of ≥ 0.1 arc s.

Speckle observations of the optical continuum emission from the central active nucleus of NGC1068 permit its angular structure from 0.03 to 0.9 arcs (2.3 to 70 pc) to be investigated. The nucleus of NGC1068 was observed with the Imperial

College of Science and Technology (London) speckle interferometer¹³⁻¹⁷ combined with the f/15 focus of the 3.9 m Anglo-Australian Telescope.

This device has an EMI, four-stage, image tube (\$20) combined with a Plumbicon TV camera and detects individual photon events, (with the combination of lenses used for NGC1068) over the image of a 3.07 arcs square ($=256 \times 256$ pixels) field. An individual recording is obtained, with an exposure time of 1/50 s, of this atmospherically distorted image which is then auto-correlated on-line within 1/25 s. Over an extended observing period these separate, two-dimensional auto-correlations are co-added and finally an auto-correlation is obtained for the 3.07 arc s² area in which objects separated by ≤ 0.9 arc s and ≥ 0.03 arc s (the limiting angular resolution of the telescope at 5,000 Å for instance) are capable of being detected. This is stored on tape in an array from -64 to +63pixels in the x dimension (right ascension) and from 0 to -63pixels in the -y dimension (declination) where the 00 pixel is the zero of the two-dimensional auto-correlation and is the centre of the narrow 'photon spike'.

The integrated auto-correlation of the field, which contains the object under investigation, is then compared with that for a star, known to be single, near in angular distance to the object and of nearly equal brightness (a neutral density filter can be used). If the observations of this reference star are made immediately after those on the object, for the same integration time and through the same filter then it can reasonably be assumed that the statistical properties of the turbulence in the atmosphere are similar in both cases and a significant comparison of the auto-correlations can be made.

The observations of the nucleus of NGC 1086 with this system are summarized in Table 1. (The 5,007 Å emission line was shown to be negligibly faint in a separate integration through a narrow filter.) Figure 1 shows a grey-scale representation (using the Manchester node of the STARLINK network) of the two-dimensional auto-correlation obtained for NGC1068 and emphasizes the very faint features (D-E) which are produced by separate sources within the 3.07 arc s² area that is auto-correlated but which are ≥200 times fainter than the single dominant source. This is primarily responsible for the intense and narrow central feature (around the 00 pixel), which is 'burnt out' in Fig. 1. However, in Fig. 2 the cut in the y direction through this central feature, from the 00 pixel, is compared with the same cut through the auto-correlation for the reference star (see Table 1). This was scaled to give the same number of counts in the photon spike around (the 00 pixel) as that obtained for NCC1068.

	Tal	ble 1 Speckle observations of	NGC1068 and refere	nce star	
Object	Field size (arc s)	Field centre (1950)	Filter (Å)	Integration time (s)	No. of separate frames autocorrelated
Nucleus NGC1068	3.07×3.07	$\alpha = 2 \text{ h } 40 \text{ m in } 07.1 \text{ s}$ $\delta = 00^{\circ}13'31.4''$	5,000-5,600	1,160	2.89×10^4
Ref. Star $(M_v = 9.0)$	3.07×3.07	$\alpha = 2 \text{ h } 41 \text{ m in } 11.33 \text{ s}$ $\delta = 00^{\circ}0'2.3''$	5,000-5,600 (ND = 1.0)	850	2.125×10^4

Table 2 Sources in the auto-correlation of NGC1068

Source	$M_{ m v}$	Separation from primary (arc s)	Separation from primary (pc)
Α	11.8 ± 0.3	0	0
В	17.7 ± 0.05	0.51 ± 0.03	40
C	19.0 ± 0.05	0.27 ± 0.03	21
D	18.2 ± 0.05	0.47 ± 0.03	37
E	17.4 ± 0.05	0.60 ± 0.03	47

The auto-correlation displayed in Fig. 1, and the cut through this and the reference star in Fig. 2, demonstrate that the nucleus of NGC1068 is dominated by a stellar-like object (source A) which is unresolved by the 0.03 arc s resolution of this system (this is a conservative upper limit to this diameter). A comparison of the counts in this central region of the auto-correlation with those obtained for the reference star give a value for source A of $M_v \approx 11.8$, which is very similar to the magnitude of the continuum emission from the entire central ≤160 pc as estimated from the work of Burbidge et al.3 and Bertola6. This suggests that most (see Table 2 and Fig. 1) of this nuclear emission originates within a region of ≤0.03 arcs diameter (≤ 2.3 pc) with a luminosity of $\simeq 5 \times 10^9 L_{\odot}$.

Several possibilities for the nature of this dominant compact source should be considered. For example, the optical emission could be intense synchrotron radiation, though the failure to detect this object using radio VLBI techniques (angular resolution of ≤0.1 arcs) could be explained by synchrotron selfabsorbtion with a turnover frequency of $\approx 10^{10}$ Hz (this implies an angular size of $\leq 10^{-4}$ arc s). Also thermal free-free absorption could be occurring in front of the source and an emission measure of only 108-109 pc cm⁻⁶ would be required to make a central synchrotron radio source not observable. However, the discovery of optical circular polarization¹⁸ argues against the synchrotron hypothesis.

Alternatively, this central source could be an extremely compact star cluster with a total mass of $\approx 5 \times 10^9 M_{\odot}$ and a mass density of $\ge 8 \times 10^8 \, M_{\odot} \, \mathrm{pc}^{-3}$ if a simplistic mass-to-luminosity relationship is applied. If this is dynamically bound then a velocity dispersion of $\ge 5 \times 10^3$ km s⁻¹ would be required which is in excess of the observed widths of the stellar absorption lines. Also, variability of the optical continuum cannot occur in the integrated light from such a large number of separate

A more likely possibility is that the compact dominant source A is a supermassive, single, condensed object comparable to that inferred to be at the centre of M87¹⁹. The existence of radio jets in Seyfert nuclei^{8,20} has been interpreted on the basis of beams emitted along the rotation axis of such objects.

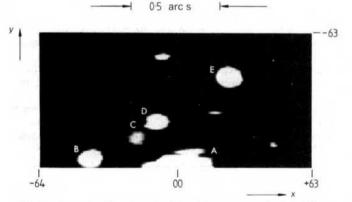


Fig. 1 A grey-scale representation of the auto-correlation of the nucleus of NGC1068. The central source A around the 00 photon spike is 'burnt out' here to permit the fainter (≥200 times) secondary sources B-E to be revealed. Other minor features are thought to be noise or cross-correlations. The irregularities around the primary A are also on the noise level and not thought to be real.

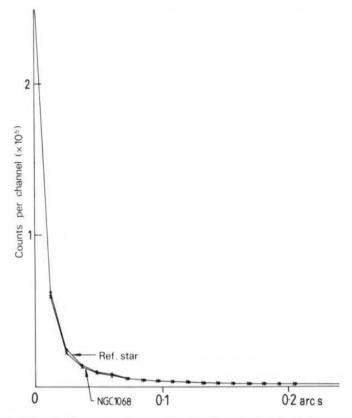


Fig. 2 The cut in the -y direction through the 00 pixel for NGC1068 in Fig. 1 is compared with the corresponding cut through the auto-correlation of the point reference star.

The secondary maxima B-E in Fig. 1 could be produced by four much fainter sources with the magnitudes and separations from the primary that are listed in Table 2. Similar features do not appear on the auto-correlation of the reference star and they are thought most probably to originate within the nucleus of NGC1068 and not be caused by faint foreground stars in our Galaxy.

However, caution is required in the interpretation of the configuration in Fig. 1 for cross-correlations between these secondary sources could also appear as secondary maxima and until some detailed modelling is carried out (which will also consider orientations of the secondary sources) it can only be stated that certainly two but possibly up to four secondary, unresolved, sources are clustered around the primary compact object in the nucleus of NGC1068. Even their nature could be unusual and require similar, but less dramatic, explanations to those for the primary source.

J.M. and H.V. thank the staff at the Anglo-Australian Telescope in November 1981, when these observations were made. This work has been supported by the SERC and in particular J.M. is grateful for his SERC Senior Fellowship.

Received 6 January; accepted 3 February 1982.

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A CCD image of the galactic centre

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Because the galactic centre lies behind approximately 27 magnitudes of visual extinction, observations at visible wavelengths are impossible. The recent introduction of charge-coupled devices (CCDs) to astronomy represents the first opportunity to use sensitive area detectors in the near IR. Using a cooled CCD, we have obtained an image of the galactic centre at an effective wavelength of 0.9 µm. Two unresolved sources were found, separated by 3 arc s along the galactic plane. The detection of the two sources was confirmed by a further observation the following night. The possibility that the new sources are foreground objects cannot be completely ruled out; however, their positions are remarkably close to those of the non-thermal radio source and the 2.2 µm source IRS-16. While it is possible that the new sources could be a pair of highly reddened individual stars or compact clusters, it seems more likely that they are two compact H II regions seen in line emission.

The CCD image (Fig. 1) was taken at the prime focus of the Anglo-Australian Telescope (AAT) on 8 September 1981 during conditions of sub-arcsecond seeing. The CCD camera was built for the Anglo-Australian Observatory (AAO) by the Royal Greenwich Observatory, and uses as a detector an RCA SID 53612 chip with 320×512 pixels, giving an image scale of 0.50 arcs per pixel. The exposure time was 10 min through an RG830 (8,300 Å long-pass) filter. Although the far-red response of the CCD is not well known, the quantum efficiency is believed to fall linearly from about 60% at ~8,000 Å to zero at 10,500 Å, thus defining with the RG830 filter an effective bandpass of ~1,000 Å centred on 9,000 Å. The image has been flat-fielded by division by an exposure of blank sky-this process is necessary to remove interference fringes which are generated in the detector chip by strong night-sky emission lines.

Comparison of Fig. 1 with a IV N Schmidt plate (courtesy of the UK Schmidt Telescope Unit) allows all the stellar images to be identified with previously known stars except for the two images marked CCD1 and CCD2. The two new sources must therefore be highly reddened as would be expected for sources in the galactic plane at the distance of the galactic centre.

In the absence of spectroscopic data one cannot completely rule out the possibility that the new sources are foreground objects which appear in line with the galactic centre purely by chance. This would require a remarkable coincidence, however, as their positions are very close to those of the non-thermal radio source and the 2.2 µm source IRS-16, which are normally assumed to mark the galactic centre. No other very red objects are prominent on the CCD frame. In addition, Allen, Carter and Malin (personal communication) have made a partial map with 2 arc s resolution of the immediate vicinity of these objects in Brackett y and find two corresponding peaks in the line intensity. Although Brackett y emission is present in Be stars, the probability of finding two such stars of almost identical magnitude within 3 arc s of a given point is infinitesimal.

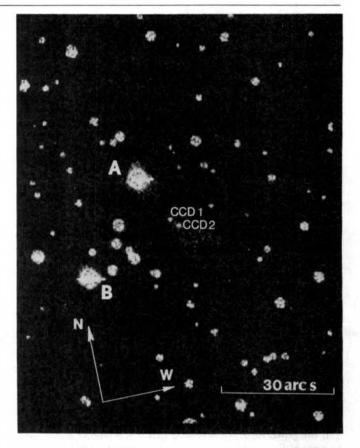


Fig. 1 CCD photograph of the galactic centre at an effective wavelength of 0.9 µm. All the stellar images are also seen on a IV N plate, with the exception of those marked CCD1 and CCD2. The bright stars identified as A and B are field stars used for positional information.

Positions for the new sources were obtained by measurement relative to two brighter visible field stars, shown as A and B on Fig. 1. These stars were in turn identified on the Palomar Sky Survey E plate and their positions determined relative to 15 nearby Smithsonian Astrophysical Observatory stars by measurement of the plate on a microdensitometer, followed by a six-parameter least-squares fit. The positions of all four stars are listed in Table 1, and should be accurate to better than 1 arc s. Star A is the visible star identified on the 2.2-μm map of Becklin and Neugebauer1; the position they determined agrees with the present value to within 0.8 arc s in each coordinate.

The intensities of the new galactic centre sources were determined by calibration against the white dwarf LDS749B, whose magnitude at 9,000 Å can be derived from the work of Oke². The resulting values are 18.9 ± 0.5 for CCD1 and 19.1 ± 0.5 for CCD2. This is equivalent to an observed flux of 1.9× $10^{-21}\,\mathrm{W\,cm^{-2}}$ from each source, corresponding to a dereddened flux of $2.2\times10^{-16}\,\mathrm{W\,cm^{-2}}$ for a visible extinction of $A_{\rm V}$ = 27 mag (ref. 3) and assuming the extinction follows Van de Hulst's curve number 15 (ref. 4).

The galactic centre has previously been detected at 1.06 and 1.18 µm using a 22 arcs aperture5. The observed magnitudes were 13.9 and 12.4 respectively. Comparison with the present result suggests that most of the flux seen by Spinrad et al.5 must have come from extended emission. There is some indication of nebulosity on the CCD frame, but its reality requires confirmation.

The shortest IR wavelength map available is that by Becklin and Neugebauer¹ at 2.2 µm. In this map, the central source (IRS-16) is seen to be slightly extended. The resolution used (2.5 arc s) is not sufficient to indicate whether the images seen by the CCD are also visible at 2.2 µm; however; they clearly are not prominent. In an early radio work at high resolution

Table 1	Posi	tions c	of galactic cent	tre sources
Object`		RA (1	950.0)	Dec (1950 0)
A B CCD1 CCD2	17 1 17 17 17	42 m 42 42 42 42	ain 30.06 s 31.63 29.53 29.47	-28° 59′ 01 2″ -28 59 27.3 -28 59 15.0 -28 59 17.4
IRS-16* Radio source†	17 17	42 42	29.3 29.335	-28 59 18 -28 59 18.60

- * Position from ref. 1.
- † Position from ref. 7.

but with limited u-v plane coverage, Balick and Brown⁶ proposed a model in which the non-thermal radio source consists of two components separated by 2.4 arcs and oriented almost north-south. Although this model has some similarity with the CCD data, further observations by Balick and Brown led them to reject it in favour of a single-component model. A more recent VLA map⁷ makes it clear that the powerful non-thermal radio source at the galactic centre does not have a double structure on the scale of arcseconds. This map (at 5 GHz) is, however, limited to a resolution of 2×8 arcs, which is insufficient to allow proper identification of the CCD images. Unfortunately, the different astrometric reference frames used at radio and optical wavelengths do not allow registration of the two maps to better than ~1 arcs. Thus one cannot rule out completely the possibility that the non-thermal radio source lies between the two newly-discovered objects, although this seems unlikely. The IR map can, of course, be precisely placed with respect to the CCD image by means of the visible field stars.

If the powerful 2.2-\(\mu\)m source IRS-7 is a late M supergiant, as is normally assumed, its apparent magnitude at 0.9 µm would be around 20. This is not much fainter than the sources detected by the CCD, and suggests that a deeper exposure would also detect this source.

If the flux from each of the newly discovered objects is due to stellar continuum at 10 kpc, then each source has a dereddened absolute magnitude in I of about -7.7. Because each image is unresolved it would be reasonable to ascribe all of this flux to a pair of individual stars. Each star would then need to be a supergiant, of spectral type K or earlier. This interpretation would be consistent with the objects being unimpressive at 2.2 µm, and with the lack of pronounced CO absorption⁸

The present results represent the shortest IR wavelength at which the galactic centre has been observed. It is therefore tempting to try to identify these objects with the ionization source for the region. Although the total flux required to power the 10 µm H II regions could be provided by a single O5 star, Lacy et al.9 have shown that this flux must be unusually soft, that is $T_{\text{eff}} \approx 35,000 \text{ K}$ (see also ref. 10), corresponding to something closer to an O9 star. It thus seems unlikely that a single pair of stars could be responsible for all the ionization. One cannot, however, rule out the possibility that the stars seen by the CCD are members of a larger cluster, whose overall ionizing output powers the region.

An alternative interpretation would be that the CCD images are due to line emission from two compact H II regions. The most plausible lines are the [S III] $^{1}D \rightarrow ^{3}P$, $J = 2 \rightarrow 1$ and $J = 2 \rightarrow 1$ 2 transitions at 9,069 and 9,532 Å respectively, with some additional contribution from the Paschen series of hydrogen. The He I 10,830 Å line would probably be too far down on the CCD response to be detectable. Assuming that all of the Brackett γ flux seen by Bally et al. 11 in an 8 arc s beam is due to the two compact sources, it is straightforward to calculate the expected [S III] flux under the assumption that the ionization state is similar to that of typical galactic H II regions. The resulting figure of 2×10^{-16} W cm⁻² is consistent with the observed, de-reddened value. Because of the very high spatial resolution of the CCD frame, it is not surprising that the two newly discovered objects are not prominent on previous, lowerresolution maps of the radio and Ne II emission.

The similarity of the two CCD images suggests that if the objects are compact H II regions, they may be ionized by a common source midway between them. Lacy et al. 12 have discussed in detail the possible existence of a $10^6 M_{\odot}$ black hole at the galactic centre. The ionizing flux from such an object would be more than sufficient to power the two compact sources found. We note that the separation of the sources $(4 \times 10^{17} \text{ cm})$ is 5,000 times greater than the expected diameter of the optically thick accretion disk of such a black hole.

The present data do not allow a unique identification of the sources detected by the CCD. It is of prime importance to obtain complete high spatial resolution maps of the region both in the 2.2 μm continuum and in Brackett γ , and to attempt to obtain spectra of the sources at 9,000 Å. This work will be attempted at the AAT as soon as possible. Meanwhile, radio maps at even higher resolution than the present VLA data would be desirable, as would improved registration of the optical and radio frames of reference. If the galactic centre sources turn out to be emitting strongly in [S III], then observations of the [S IV] line at 10.5 µm would yield a precise determination of the temperature of the ionizing radiation field. This would, in turn, place tight constraints on the nature of the ionizing source.

We thank the director of the AAO for allocating us time during the commissioning of the CCD to make these observations, D. Carter for help with the data taking and reduction, R. G. Dean for rescuing the data tape, D. A. Allen and P. Murdin for helpful discussions, and A. E. T. Schinckel for careful-measurement of the Palomar plate.

Received 22 December 1981, accepted 11 February 1982

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A simple ice sheet model yields realistic 100 kyr glacial cycles

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Records of global ice volume for the past 700 kyr, based on oxygen isotopic data from deep-sea cores and reflecting mainly the changing Northern Hemispheric ice sheets, show a dominant cycle of roughly 100 kyr period. The records also show smalleramplitude oscillations with spectral peaks at roughly 40 and 20 kyr periods, which are well correlated with the Milankovich insolation variations due to perturbations in the Earth's orbital parameters. However, no model has accurately simulated the 100 kyr glacial cycle. Recently Birchfield et al. and Oerlemans have obtained encouraging agreement with some features of the glacial cycle by using a simple ice sheet model with a realistic time lag in the response of the bedrock to the ice load. This study extends their basic model, first by including topography to represent high ground in the north. Improved results can then be obtained but only with unrealistic parameter values and for some aspects of the record. Further improvements are

obtained by crudely parameterizing possible calving at the equatorward ice sheet tip during deglaciation by proglacial lakes and/or marine incursions from the Atlantic, as emphasized by Andrews³. The resulting ice volume curves agree fairly well with the observed records and their power spectra over the past 700 kyr.

The ice sheet model^{1,2} predicts ice thickness in a cross-section running approximately north-south along a typical flow line (see Fig. 4). A vertically integrated approximate ice flow law is used with east-west flow neglected, which reduces the ice dynamics to a non-linear diffusion equation for ice thickness h

$$\frac{\partial h}{\partial t} = A \frac{\partial}{\partial x} \left[h^{\alpha} \left| \frac{\partial (\dot{h} + h')}{\partial x} \right|^{\beta} \frac{\partial (h + h')}{\partial x} \right] + G(h + h', x, \text{ orbit})$$
(1)

where t is time and x is distance to the south. h' is the elevation of the bedrock surface above a fixed reference level (taken as the present mean sea level). For all results below $A = 5.77 \times 10^{-4} \,\mathrm{m}^{-3} \,\mathrm{yr}^{-1}$, $\alpha = 5$ and $\beta = 2$. The northern boundary of the model is taken at 74° N to represent the Arctic Ocean shoreline, where h is required to be zero (this excludes the possibility of marine ice sheets forming in the Arctic⁴). G is the net annual mass balance on the ice surface and crudely represents the current distributions of snowfall and ice melt. Its dependence on the surface elevation h + h' follows Oerlemans²:

$$G = \begin{cases} a(h+h'-E) - b(h+h'-E)^2 & \text{if } h+h'-E \le 1,500 \text{ m} \\ 0.56 & \text{if } h+h'-E > 1,500 \text{ m} \end{cases} \text{ m yr}^{-1}$$

where $a = 0.81 \times 10^{-3} \text{ yr}^{-1}$, $b = 0.30 \times 10^{-6} \text{ m}^{-1} \text{ yr}^{-1}$. At elevations below the equilibrium-line altitude E, G becomes rapidly negative (due mainly to summer air temperatures being above freezing), whereas above E, G increases more slowly to a maximum value. As in previous studies, E is assumed to have a constant slope in latitude and to be shifted uniformly by the insolation variations according to $E = E_0(x) + k\Delta Q$, where E_0 is the present equilibrium line and ΔQ is the difference in the summer half-year insolation at 55 °N from that of the present, calculated from the orbital parameters⁵. E_0 and the insolation sensitivity, k, are specified for each run below.

Fig. 1 Total cross-sectional area of ice versus time for various model versions (solid curves). The corresponding approximate ice volume can be obtained by multiplying by a typical east-west ice sheet dimension 3,000 km). The dotted curve in each panel is an oxygen isotope deep-sea core record minus its present value, redrawn from ref. 20. a, Model run with no topography $(h'_0(x) =$ 0) and with the bedrock in isostatic equilibrium $(\nu = \infty, h' = -rh)$; the present equilibrium line $E_0(x)$ passes through the (lat. (°N), height (m)) point (73, 0) with a slope of 0.9× 10^{-3} ; the insolation sensitivity $k = 25 \text{ m ly}^{-1}$ day; the ice/rock density ratio $r = 10^{-3}$ 0.3. b, As a except with bedrock lag $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$ (in this and subsequent model runs, the maximum ice areas attained correspond to southern tip positions of ~49-52 °N). c, Model run with piecewise-linear topography $h'_0(x)$ joining the following (lat., height) points: (72, 0), (70, 850), (66, 200), (40, 400), (30, 400); the northern model boundary is at 72 °N; $E_0(x)$ passes through (66, 850) with a slope of 1.0×10^{-3} ; $k = 20 \text{ m ly}^{-1}$ day; $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$; r = 0.4. d, Model run including the calving mechanism (equation (4)); $h'_0(x)$ joins (74, -500), (70, 850), (66, 200), (40, 400), (30, 400) (the calving mechanism operates permanently at the northern tip,

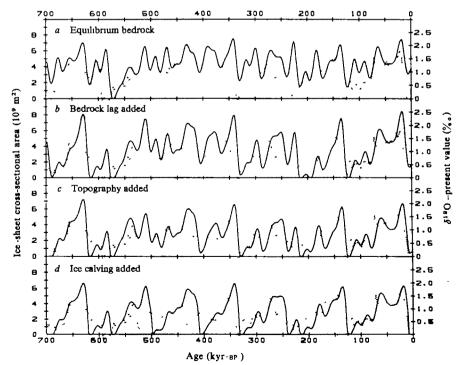
The response of the bedrock to the changing ice load consists of an elastic deformation of the lithosphere and a deeper viscous flow in the asthenosphere. As in other models^{1,2} the smaller elastic deformation is neglected here. It is not generally agreed whether the best asthenospheric model is closer to a relatively thin channel or a deep half-space⁶⁻⁸; the thin-channel model is used here, which with linear viscosity yields^{6,9}

$$\frac{\partial h'}{\partial t} = \nu \frac{\partial^2}{\partial x^2} [h' - h'_0(x) + rh] \tag{3}$$

where r is the ratio of ice density ρ_1 to rock density ρ_* ; ν is $\rho_*gH^3/N\eta$ where g is the gravitational acceleration, H is the mean thickness of the channel, η its dynamic viscosity, and N is 12 or 3 (depending on whether zero tangential velocity or zero tangential stress is required at the base of the lithosphere). $h'_0(x)$ is the surface topography due to crustal structure that would prevail in isostatic equilibrium without any ice. The choice of lateral boundary conditions for equation (3) is uncertain; for the runs below it was required that $h' = h'_0$ at the model boundaries 74°N and 30°N, but other reasonable choices have little effect on the results. The main effect of using equation (3) instead of a local-response equation^{1,2} is that the bedrock adjusts rapidly to small-scale ice loading, whereas the broad deep depressions under the largest ice sheets can be preserved for a much longer time during subsequent deglaciations.

Equations (1) and (3) were numerically integrated forward in time using a Newton-Raphson scheme for each equation individually, with an implicit contribution from every term involving h in the ice flow expression in equation (1). This allowed a time step of 50-100 yr with a latitudinal spacing of 55.5 km (= 0.5° lat.). Tests were made to check that the results agree adequately with those at higher resolutions. Many runs were made varying the parameters E_0 , k, r, ν and $h'_0(x)$, and the results are summarized in Fig. 1. For comparison a deep-sea core record is shown in each panel (dotted curve); this is interpreted here as being proportional to Northern Hemispheric ice volume, although it may also have been affected significantly by Antarctic ice sheet variations 10.

Figure 1a shows a run with no topography $(h'_0(x) = 0)$ and with the bedrock always in isostatic equilibrium with the ice load $(\nu = \infty$, that is h' = -rh). This is similar to results of earlier



maintaining it at ~72.5° N); $E_0(x)$ passes through (70, 550) with a slope of 1.0×10^{-3} ; $k = 17 \text{ m ly}^{-1}$ day; $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$; r = 0.3; sea level S = 0 in equation (4).

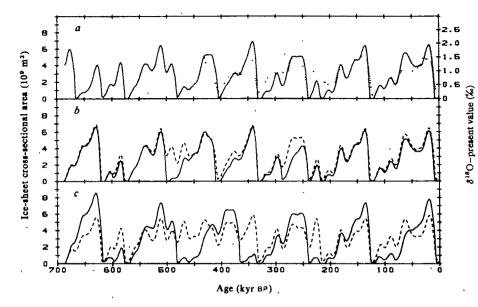


Fig. 2 a, Solid curve, as Fig. 1d except sea level $S = -100 \times$ (ice cross-sectional area/ 6×10^9 m²) m, and with different initial conditions. Dotted curve, oxygenisotopic deep-sea core record minus its present value, from the cores RC11-120 and E49-18, redrawn from ref. 29. b, Solid curve, as Fig. 1d except sea level S = 100 m. Dashed curve, as Fig. 1d except sea level S = -100 m. c, Solid curve, as Fig. 1d except $\nu = 30 \text{ km}^2 \text{ yr}^{-1}$. Dashed curve, as Fig. 1d except $\nu = 300 \text{ km}^2 \text{ yr}^{-1}$.

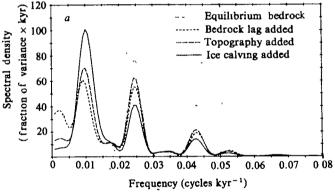
plastic ice sheet models^{11,12}, adequately reproducing the observed 20 and 40 kyr oscillations but with no trace of a 100 kyr cycle. The main features that this model lacks are the rapid retreats from glacial to interglacial conditions, for instance the last deglaciation from \sim 18 to \sim 6 kyr BP. These retreats are responsible for the asymmetric 'sawtooth' shape of the 100 kyr cycle and may even be essential to its existence. If the magnitude of the orbital forcing is increased in this model, the amplitude of the oscillations simply increases and also the ice sheet can vanish for extended periods, as in the earlier plastic models, however, no real resemblance to the observed glacial cycle can be obtained.

Figure 1b is produced with no topography but with $\nu = 100 \,\mathrm{km^2\,yr^{-1}}$, corresponding to a bedrock lag time of $\sim 10 \,\mathrm{kyr}$ for the broadest features. This curve is similar to that in ref. 1, with the bedrock lag producing some rapid deglaciations or partial retreats by the following mechanism: at a glacial maximum the bedrock depression is relatively deep, so that any subsequent retreat is amplified by the increased ice melt at the lower surface elevations near the southern tip. These retreats are initiated at times of high summer insolation and coincide well with some of the observed deglaciations; however, there is still only partial agreement with the observed records. One drawback is that to achieve the retreats, the amplitude of the orbital forcing, k, has to be set unrealistically large which results in too much power at high frequencies (see Fig. 3a).

Another disconcerting feature of Fig. 1b is that some of the retreats do not appear to be complete enough; for instance, the present day model ice sheet extends from the Arctic shoreline to 65.5° N, and during the period 550-350 kyr BP the southern tip never retreats beyond 65° N. However, it is not known exactly how far the real ice sheets retreated in earlier interglacials; the minimum δ^{18} O values for these times in various deep-sea cores are sometimes less and sometimes greater than today's value. Ice volumes can also be inferred from eustatic changes in sea level measured by raised coral reefs, and during the last interglacial at ~125 kyr BP the sea level was ~5 m higher than at present13, suggesting that the Northern Hemisphere was at least as ice-free as today. Unfortunately, sea-level estimates for the earlier interglacials are more uncertain due to tectonic uplift, and show only that the ice volume must have been a small fraction of that during an ice age¹³.

At this point the model seemed to need more potential for complete deglaciations, but with less amplitude of the orbital forcing. This was attempted first by including topography in the model. The high ground on the Labrador-Ungava Plateau and/or Baffin Island has often been considered as important for the inception of ice sheets, and has been included in several

recent models ¹⁴⁻¹⁶. Figure 1c shows a run with the topographical term h'_0 in equation (3) roughly representing a present day section running south-southwest from Baffin Bay, over the high ground on Baffin Island, through Hudson Bay and on into the central USA; added to this is the residual depression from the last glaciation of up to 300 m centred on Hudson Bay¹⁷. The most important feature is the Baffin Island plateau with a peak at 70° N, and similar results can be obtained with this feature alone. By carefully adjusting the mean equilibrium line E so that it occasionally intersects the peak, small ice sheets can



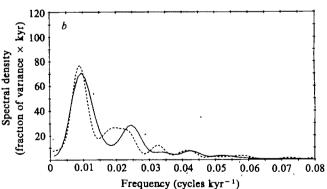


Fig. 3 a, Power spectral density for the model runs shown in Fig. 1. These were generated from time sequences of 350 points taken from each run at 2 kyr intervals, and detrended with a linear least-squares fit before computing autocovariances. A Tukey window was used with the number of lags $=\frac{1}{3}$ of the total number of points. b, Power spectral density for the two deep-sea core records shown as the dotted curves in Figs 1 and 2a. These were computed as for the model curves above. Dashed curve from ref. 20; solid curve from ref. 29.

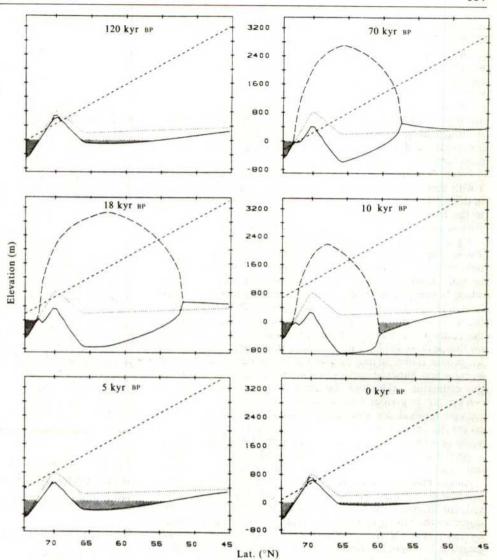


Fig. 4 Cross-sections versus latitude and height showing the ice sheet surface (long-dashed line) and the bedrock surface (solid line) at various times in the model run of Fig. 1d. Also shown are the equilibrium line E (short-dashed line) and the undisturbed topography h_0' (dotted line). The shaded regions show the extent of the invading water body that attacks the ice according to equation (4).

easily be initiated and grow out from the peak, whereas to the south E can still be high enough to place large ice sheets in jeopardy of complete deglaciations. Although the curve shows some improvement between ~ 350 and 0 kyr BP, the greater deglaciations were achieved only after careful tuning, especially in matching E to the topography. In addition an unrealistically large value for the ice/rock density ratio, r=0.4, was required, and the required amplitude of the orbital forcing, k, still results in too much high-frequency power (see Fig. 3a). Even so, the period from 550 to 350 kyr BP still contains no complete deglaciations. Also the growths out of the interglacials at ~ 330 and 215 kyr BP are unrealistic, with anomalous partial retreats occurring at 285 and 170 kyr BP respectively.

These shortcomings indicated that an additional mechanism was required to yield a more robust model. It was noted that during the retreats in Fig. 1c coinciding with the observed deglaciations the southern tip of the ice sheet temporarily dropped far below sea level. Andrews3 has emphasized that rapid calving by vast proglacial lakes, which are known to have existed around the southern Laurentide ice sheet perimeter during the last deglaciation, may be the only plausible mechanism capable of producing the observed rapid rates of retreat. This view is supported by deep-sea core evidence of Ruddiman and McIntyre 18, who infer massive discharge of icebergs into the North Atlantic after ~16 kyr BP. In addition marine incursions up the St Lawrence river and later into Hudson Bay at -8 kyr BP are known to have greatly accelerated the retreat3. The calving process was included in the model by setting the mass balance G equal to a large negative value at ice sheet points between the tip and the first interior ice sheet grid point

able to block the invading body of water. The invading water, whose surface is taken to be at sea level, can penetrate into the ice sheet only as far as the ice columns can be floated by its hydrostatic head, so that

$$G(x_{i+1}) = -20 \text{ m yr}^{-1} \text{if} \qquad \boxed{\rho_1 h(x_i) < \rho_w(S - h'(x_i)) \\ \text{and } h'(x_{i+1}) < S}$$
(4)

where ρ_w is the density of sea water, S the elevation of the current sea level and i the spatial grid index increasing into the ice sheet. Condition (4) is generally only satisfied, if at all, at the outermost $\sim 50-150$ km of the ice sheet when the tip is below the level S. The arbitrary value of -20 m yr⁻¹ in equation (4) is comparable with estimates by Andrews³, but is much less than those suggested by analogies with non-floating calving glaciers today¹⁹.

Figure 1d shows a run including this calving mechanism and with the topography described above. Deglaciations, now augmented by equation (4), can occur with a reduced amplitude of the orbital forcing, and the overall agreement with the observed record is improved considerably. Interestingly the high insolation anomaly that triggers the deglaciation at 410 kyr BP is considerably weaker than those at some other times, for example around 175 and 85 kyr BP. Deglaciations do not occur at the latter times because the ice sheet is too small and the bedrock surface too high to allow calving.

There are still some disagreements with the observed records, especially before ~400 kyr BP; however, before this time the observed records themselves tend to diverge from each other 1,20. In fact, the apparent agreement can be improved by

selecting another deep-sea core record for comparison, as shown in Fig. 2a. For this model run the sea level S was made a linear function of ice volume and different initial conditions were chosen to show the relative insensitivity of the model with calving (in fact, much the same results can even be obtained with less realistic flat topography southward of 72° N). The effects of varying most of the model parameters have been investigated, and two examples are shown in Fig. 2b and c. Although these parameter changes are fairly substantial, the occurrence and phase of most of the main glacial cycles are preserved.

Figure 3 compares the spectra for the model curves in Fig. 1 with those of the deep-sea core records. A gradual increase in the ~100 kyr peak is seen as each new mechanism is added to the model. The spectrum with the calving mechanism has relative peak sizes in good agreement with those observed.

Figure 4 illustrates the last glacial cycle of the run in Fig. 1d. The ice sheet appears first on the northern peak at 120 kyr BP, then grows out to its maximum size which makes it susceptible to rapid deglaciation triggered by a period of high insolation, which begins just after 18 kyr BP. The calving mechanism sets in at ~ 11 kyr BP when the ice sheet tip is at 57° N, and enables the ice to vanish completely just after 5 kyr BP. By the present the bedrock has rebounded enough to support a small ice cap on the peak at 70° N. This is smaller than the present Barnes ice cap on Baffin Island, despite the model equilibrium line E being a few hundred metres below that suggested by present day estimates²¹. Although the amount of bedrock uplift since ~8 kyr BP is greater than observed by factors of ≤ 2 , the present residual depression agrees roughly with that implied by gravity anomalies¹⁷, and the present region below sea level south of ~66° N corresponds roughly with that of Hudson Bay.

As the simple model used here is tested mainly against just one type of observed record, any positive results should be regarded mainly as a guide for future work. Many feedback processes involving other parts of the climate system are neglected; for instance, the improvement in the model curves produced by the calving mechanism might also have been produced by any highly ablative mechanism that can be triggered only after the ice sheet has reached maximum size. One example is the freshening and shallowing of the oceanic mixed layer by massive ice sheet runoff, a self-amplifying mechanism thought primarily to reduce snowfall in winter 22 and recently supported by deep-sea core evidence²³.

Equation (4) is an extremely simplified parameterization of the complex and not well understood mechanism of calving. An improved treatment would probably require the inclusion of east-west topography and ice sheet structure; with the present model equation (4) cannot distinguish between the known marine incursions from the east (up the St Lawrence river channel and into Hudson Bay, around each side of the Labrador-Ungava plateau), and the inland proglacial lakes that formed around the retreating Laurentide perimeter. Marine deposits from the past several thousand years are found only in the vicinity of the St Lawrence and the shores of Hudson Bay^{3,17}. This implies that the inland proglacial lakes formed mostly above sea level, in apparent disagreement with the model whose equatorward tip consistently drops a few hundred metres below sea level during deglaciation (Fig. 4). However, the basic mechanism represented by equation (4) is known to have induced rapid ice sheet disintegration when the sea invaded Hudson Bay at ~8 kyr BP (refs 3, 24), and is suspected of having induced past disintegrations of the West Antarctic ice sheet²⁵. The scenario hypothesized here could also apply to the Fennoscandian ice sheet, where the Scandinavian mountains form a northern plateau for ice sheet inception and the low-lying Baltic regions could have allowed the calving mechanism to be active during deglaciation.

Possible further work includes an extension of the model to three dimensions^{15,26}, improved treatment and comparison with data of calving by water bodies after ~16 kyr BP (refs 3, 18), inclusion of the elastic response of the lithosphere²⁷, and a test of the results using a deep half-space asthenospheric model²⁸. In addition equation (2) can be replaced by coupling to a global seasonal climate model^{12,16}.

This research was supported by the Climate Dynamics Section of the NSF under grant ATM-8019762. I thank the staff at the Climatic Research Institute, Oregon State University for computing support, and J. T. Andrews, G. E. Birchfield, W. F. Ruddiman and L. D. Williams for helpful comments.

Received 5 October 1981, accepted 24 January 1982

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Interaction of cryospheric forcings with rotational dynamics has consequences for ice ages

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Recent geological evidence^{1,2} suggests that several degrees of true polar wandering have occurred since the mid-Pliocene. This rotational instability is supported by dynamical calculations3 of the spin axis of a layered viscoelastic Earth, which has been subjected to the periodic forcings characteristic of the late Cenozoic ice age. We propose here that polar wandering, induced by the intrinsic response of the viscoelastic planet to such cryospheric forcings, may be the underlying cause for the termination of the present ice age epoch.

In the past decade there has been an increasing number of observations4-7 based on the correlation between the periodicities of the Earth's orbit and the time series analyses of the oxygen isotope data to suggest that there is a cause and effect relationship between the two systems. Nonetheless, it has been argued that internal stochastic mechanisms⁸⁻¹⁰ could be an important source for climatic variations. A new generation 11-14 of climatic models has also emphasized the need to include the non-linear response of an ice sheet to changes in solar radiation in the attempt to understand better the mechanisms of ice ages. Here we pursue this theme and focus attention on the long-term effects of cryospheric forcings on producing secular motions of the Earth's spin axis, more commonly known as true polar wandering (TPW). The important consequences of TPW on the evolution of the climate system over a long time scale had long been appreciated 15 and Gold 16 had proposed that polar wandering is not only physically feasible but is to be expected for a viscoelastic planet.

3

First, we consider the recent findings 1-3 that suggest that there may be a connection between the late Cenozoic glaciation and TPW of ~5° in the past 10 Myr, as revealed by the recent reanalyses of the paths of palaeomagnetic poles. These data are based on the reconfirmation that hotspots remain relatively fixed with respect to one another for the past 150 Myr. Calculations based on a layered viscoelastic Earth have supported this hypothesis that such an amount of polar wander relative to the entire mantle could, in fact, have occurred in response to the waxing and waning of large ice sheets in the past few million years. Over a shorter time scale, evidence¹⁷ from the past 75 years of International Latitude Service data also shows that TPW is taking place today. This phenomenon can be shown to be the direct consequence of the late Wisconsin deglaciation on the Earth's rotation 18,19. Thus evidence is accumulating to the effect that cryospheric forcings can strongly influence polar motions for a wide range of time scales. Our model calls on polar wander induced by ice ages ultimately to exceed a critical value of angular displacement which will dislodge the present climatic trend from its apparent equilibrium configuration. Such a finite-amplitude perturbation in the amplitude of rotation may cause the termination of ice ages. This event cannot be explained by Milankovitch's theory of astronomical forcings.

We now present our model which provides a physical descrip-

tion of polar wander produced by glaciation.

The expression of the conservation of the system angular momentum associated with the cryosphere and solid Earth is given by the Eulerian equations of motion, more commonly known in geophysics as the Liouville equation²⁰. For forcing functions from glacial loadings it takes the vector form

$$\frac{\mathrm{d}}{\mathrm{d}t}(\mathbf{J}\cdot\boldsymbol{\omega}) + \boldsymbol{\omega}\times\mathbf{J}\cdot\boldsymbol{\omega} = 0 \tag{1}$$

which is applicable for a body-fixed coordinate system rigidly attached to the Earth as a whole with its origin at the centre of mass and with the axes oriented such that the inertia tensor J initially is diagonal. ω is the angular velocity vector.

The J tensor as a function of time t may be decomposed as follows.

$$\mathbf{J}(t) = I\delta_{\mathbf{u}} + C_{\mathbf{u}}(t) + I_{\mathbf{u}}(t) \tag{2}$$

where I is the diagonal inertia tensor of a bulging Earth in the absence of rotational fluctuations, $C_q(t)$ is the component of the inertia tensor due to changes in the basic rotation rate, and $I_q(t)$ is the contribution produced by transient viscous flow in the mantle and by shape deformation due to the glacial forcings. Variations in $I_q(t)$ drive the spin axis away from its original position, whereas $C_q(t)$ acts as a stabilizing agent in the readjustment of the rotational bulge as a consequence of the sudden change in the moment of inertia.

The nonlinear version of the Liouville equation must be used for polar displacements >20° (ref. 19). But for the amount of polar drift found in the past 10 Myr (refs 1, 2) the linearized Liouville equations may be employed with sufficient accuracy. The solution has the simple Laplace transform domain form

$$\mathbf{m}(s) = \frac{\psi(s) + \phi(s)}{\left(1 + \frac{i}{\sigma_r} s\right)}$$
(3)

where $\mathbf{m} = (m_1, im_2)^T$ are the directions cosines of the rotation axis in the body-fixed coordinate system, s is a complex number and σ_r is the Chandler wobble frequency for the rigid Earth. $\psi(s)$ and $\phi(s) = (\phi_1, i\phi_1)^T$ respectively are the forcing functions arising from rotational deformation and the surface loading from ice masses. They have the explicit forms

$$\psi(s) = \frac{k_2^T(s, a)\mathbf{m}(s)}{k_t} \tag{4}$$

$$\phi_1(s) = \frac{I_{13}(s)}{C - A} + \frac{(sI_{23}(s) - \bar{I}_{23})}{\Omega(C - A)}$$
 (5)

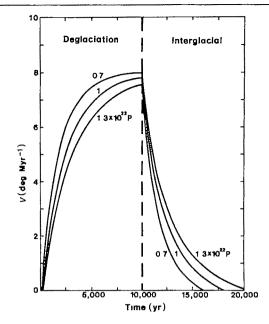


Fig. 1 Instantaneous speed of polar wandering during a glacial cycle. The length of time of the deglaciation phase is taken to be 10⁴ yr, characteristic of the late Wisconsin. The speed of polar wander is taken to be positive when the direction of motion is towards the glaciating area. The end of the last deglaciation phase is around 5-6 kyr BP.

$$\phi_2(s) = \frac{I_{23}(s)}{C - A} - \frac{(sI_{13}(s) - \overline{I}_{13})}{\Omega(C - A)}$$
 (6)

where Ω is the diurnal rotation rate, C and A are the axial and equatorial moments of inertia respectively and the overbars denote quantities at time t=0. $k_2^T(s,a)$ is the tidal Love number, which represents the time-dependent behaviour of the gravitational potential in response to fluctuations in the rate of rotation, for angular order l=2 evaluated at the surface (r=a) and k_l is the fluid Love number, which is a measure of the Earth's yield to centrifugal deformation over a time scale $0 (10^9 \text{ yr})$. Its value is about 0.95 (ref. 20).

For a given Earth model, $k_2(s, a)$, $I_{13}(s)$ and $I_{23}(s)$ can be obtained spectrally from the solution of a boundary value problem. Using a three-layer model, which consists of an elastic lithosphere, a viscoelastic mantle, and an inviscid core, we have obtained analytical expressions³ for the spectral decomposition of equations (4)–(6), which greatly facilitates the solution of the initial value problem of the polar displacement as a result of glacial forcing.

This three-layer model contains the essential physical ingredients needed for polar wander to occur. (1) The lithosphere is not isostatically compensated in the final state. Thus this residual elastic strain field produces a net polar displacement after the completion of a glaciation cycle. (2) Transient viscous flow in the mantle is influenced by the core and thereby produces another relaxation mode, whose strength of excitation is of the same order as that associated with the mantle mode.

The best available data which provide information on the actual time dependence of the extent of Pleistocene glaciation are the time series of oxygen isotope variability^{5,21}. To show that rapid rates of polar wander can occur at the glacial-interglacial boundary, we have conducted a series of model calculations for contributions from the two major ice sheets in the Northern Hemisphere, Laurentide in North America and Fennoscandia in Europe. A total mass of 2.4×10^{19} kg has been used in the loading and unloading process. For mathematical convenience a ramp-shaped function with an accretion time of 9×10^4 yr and with a disintegration time of 10^4 yr has been used to describe the climatic fluctuations³. This time-dependence can be transformed readily in the Laplace transform domain and used in equation (3). Figure 1 shows that with a range of

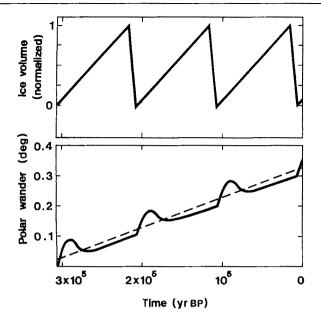


Fig. 2 Polar wander caused by cryospheric forcings. The forcing function is given at the top by the time-dependence of the ice volume, which has been normalized by an ice volume which is equivalent to a 70-m rise of the global sea level, a representative value of the unstable ice sheets in the Northern Hemisphere³³. The time of termination of the last deglaciation phase is taken to be 6 kyr BP. The response of the rotating viscoelastic Earth to such forcings takes the form of polar wandering. During this period the dashed line stands for the long-term polar displacement, whereas the solid line represents the short-term polar movements in response to glaciation and deglaciation.

mean mantle viscosities $0 (10^{22}P)$ quite rapid rates of polar wander, up to 8° Myr⁻¹, which translates into around 80 cm yr⁻¹, can take place. However, as the period of deglaciation, $0 (10^4 \text{ yr})$, is short, the total polar displacement is only about 10 km. This amount, which involves polar motion relative to the mantle, is much smaller than changes in the obliquity, which involves polar motion relative to the fixed stars. This latter phenomenon arises from an external torque and may amount to 3-4°. Therefore, we would not expect our sporadic rapid motions of 80 cm yr⁻¹ to have much of a visible signature on the ice coverage record. However, these rapid secular changes of the rotation axis may have some unforseen effects on climatic variations²² between 10^3 and 10^4 yr.

Given that such rapid polar wander can occur in response to a single glaciation cycle, what then are the long range prospects for the late Cenozoic ice ages, which from the proxy climatic record seem to have reached a quasi steady-state trend consisting of a saw-tooth pattern for the past million years 5.23,24? To assess this possibility we have calculated the amount of polar wander, which has occurred since the mid-Pliocene from a forcing function which has been constructed on the basis of the proxy climatic record 5.23. Our calculations indicate that an average polar wander speed of 1° Myr⁻¹ is attained for a viscosity of 10²²P.

These modelling results suggest that cryospheric forcings must be capable of producing TPW because of the basic physics due to the Earth's viscoelastic rheology¹⁶. If the present pattern of ice age cycles remains unchanged, then, barring any sudden changes of present plate motions, which might counteract the effects of TPW, Antarctica would begin to drift northward gradually by virtue of TPW. At the initial stages of this secular drift climatic warming may cause an increase of the melting from the Antarctic ice shelves. Over a short time scale, 0 (10²-10³ yr), continued weakening of the ice shelves²⁵ would then result in the ultimate collapse of the weakened portion of the Antarctic ice sheet with a consequent worldwide rise in the sea level of at least a few metres.

In the prevailing conditions of the Cenozoic ice ages, the entire mantle would shift relative to the spin axis. In this connection, data which have been combined from equatorial sediment facies²⁶ and palaeomagnetism²⁶, strongly support the occurence of TPW, at least since the Cretaceous. They yield a motion of the rotation axis with a direction of about 25°E (towards Eastern Europe) in a reference frame in which the hotspots are fixed^{1,26}.

Secular motion of the Earth's spin axis relative to the geographical distribution of oceans and continents can have a profound influence on the future trend of the current ice age cycles. Milankovitch²⁷ considered that variations in the orbital configuration are critical for rapid growth of ice sheets in the Northern Hemisphere. The magnitude of the orbital variations are of a few degrees^{28,29}. Hence, if the present trend of polar wander were to exceed a certain threshold value, 0 (10°), then such a large perturbation may bring about drastic changes in the climate system, such as changes in the role of the Coriolis forces, which control the patterns of oceanic currents to a large extent. Situated adjacent to the largest Northern Hemisphere ice sheets of the ice ages, the mid-latitude North Atlantic Ocean has, indeed, an important role in the Earth's recent climate history³⁰. One would expect, therefore, that under these circumstances of a shift in the direction of the Gulf Stream the response of the climate system to orbital variations might be altered drastically.

To demonstrate the feasibility of this mechanism, we have calculated in Fig. 2 the amount of TPW due to the periodic forcings from ice ages in the past 3×10^5 yr. For a mean mantle viscosity of 10²²P, the speed of polar wander is about 1° Myr⁻¹. The saw-tooth curve of the Quaternary Ice Age shows clearly a 10⁵-yr quasi-periodicity^{21,23}. The occurrence of an increasing trend in polar wandering in response to continual cryospheric forcings is a natural consequence of the rheological behaviour of the Earth. The time scale for this quasi-steady period is 0 (10 Myr), if the average polar wander speed is maintained steadily at 0 (1° Myr⁻¹) as shown in Fig. 2. Angular displacements of this magnitude would seem sufficient to displace the present configuration of continents and ocean circulations from the state of quasi-equilibrium which it now seems to enjoy. From then this new set of surface boundary conditions will not act in concert with astronomical forcings to produce large scale continental glaciation.

Finally, for each epoch of ice ages, the magnitude of the cryospheric forcings is governed by the particular geographical distribution of continental platforms, which may be linked with the possible time-dependent nature of mantle convection. For the Cenozoic ice age, whose inception is still fraught with uncertainties^{31,32}, we may expect this present periodic phase to persist for another few million years, before it undergoes self-destruction by TPW.

This work was supported by the Antarctic Research Division of the NSF, the Research Corporation and the Progetto Finalizzato Geodinamica.

Received 24 November 1981, accepted 27 January 1982

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Picrites as parental magma of MORB-type tholeiites

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Recent experimental work on peridotites and basalts 1-3 and a reappraisal of ophiolite complexes4 supports the concept that basalts are derived from picritic parents⁵⁻⁷. The occurrence of a density minimum in the liquid line of descent⁸⁻¹⁰ explains why mid-ocean ridge tholeiites fall in a restricted compositional range, and parental picrites can only be found in the exceptional case when eruption is not yet controlled by a steady-state magma chamber^{7,11}. We present here a preliminary account of a natural example of such a case from the Caribbean.

The Cretaceous Curação Lava Formation is a more than 5-km thick succession of submarine basalts with more than 20 flows of picritic pillow basalts in its lower part. The base of the succession is not exposed. The picrites are associated with variolitic basalts and olivine-phyric tholeiites. Olivine-phyric and plagioclase-pyroxene-phyric tholeiites make up the bulk of the formation, the latter predominating in the upper part. Composition of glass and of olivine megacrysts and phenocrysts in the picrites indicates that the olivine-phyric tholeiites were derived by fractionation of olivine from a picritic parent with at least 17.5 wt% MgO. The plagioclase-pyroxene-phyric tholeiites are derived by further fractionation of olivine, plagioclase and pyroxene. The picritic basalts in the section have 19-31 wt% MgO, which shows that part of their olivine is cumulative. Model calculations suggest that the parent results from 30% partial melting of a mantle of pyrolitic composition.

The variolitic basalts associated with the picrites in the lower part of the unit, pose a problem. Major element chemistry of these rocks show that they are not related to the other basalts by low pressure fractionation. However, incompatible element ratios are roughly similar for all basalts of the succession, which suggests that they are derived from the same mantle source.

The island of Curação (positioned between 12°2' N and 12°23'30" N, and 69°10' W and 68°44'30" W) is the larger of an east-west-trending group of small islands in the southern Caribbean. Geophysical and geological evidence suggests that the islands are underlain by a basement of oceanic crust of pre-Campanian age¹². The Curação Lava Formation is the lowermost unit exposed in Curação 13. It consists almost entirely of volcanic rocks but contains one intercalation of a few metres thickness of pelagic sediments in its upper part. These contain an ammonite fauna of middle Albian age¹⁴. The Curação Lava Formation consists largely of pillowed flows. They are virtually the only rock-type in the lower half of the formation; in the upper half they are still predominant, but alternate with reworked hyaloclastites and dolerite sills and dykes. A sheeted dyke complex is not exposed.

Whole-rock major elements used here have been determined by X-ray fluorescence spectrometry (University of Amsterdam); rare earth elements (REE) by instrumental neutron activation analysis (Inter-university Reactor Institute, Delft); glass and minerals were analysed with an electron microprobe (Instituut voor Ardwetenschappen, Vrije Universiteit, Amsterdam).

Most picrites contain between 19 and 22 wt% MgO (Fig. 1). These flows all have pillow structure and vary in thickness from a few tens of metres to roughly 100 m. Pillow shape and size is equal to those of the tholeiites. The rocks consist of more or less euhedral olivine phenocrysts (Fo_{86.5-87.4}) of up to 2.5 mm in size, embedded in a groundmass of glass and skeletal quench crystals of olivine and clinopyroxene. The amount of glass decreases rapidly from rim to core of the pillows. Chrome-rich spinel occurs as an accessory constituent. The olivine phenocrysts are unzoned. They make up 27-32 vol.% of the lavas. Gravitational settling of olivine within a flow or within a single pillow is negligible, and the proportion of olivine always falls within these limits. Glass from the pillow margin of one of these picrites (79KV615, Table 1) plots on the Fo₈₇ control line and within the field of the olivine-phyric tholeiites (Fig. 1). This glass (MgO = 10.6%, FeO = 8.6%) is similar in composition to the most primitive liquids erupted at mid-ocean ridges. It is in equilibrium with the Fo_{87} phenocrysts, using a K_D for partitioning Mg and Fe between olivine and melt of 0.3 (ref. 15). This implies that the olivine-phyric tholeiites are derived from a parent with a MgO-content ≥10.6 by equilibrium crystallization of olivine. The occurrence of strongly resorbed olivine mega-

Table 1 Representative analyses of picrites and basalts of the Curação Lava Formation

	Picrite 79KV615	Glass picrite 79KV615	Olivine phenocryst 79KV615	Olivine-phyric tholeiite 79Be248	Plagioclase- pyroxene- phyric tholeiite Be2041	Variolitic basalt 79BK207
SiO ₂	45.83	49.7	40.6	50.61	51.22	51.46
TiO ₂	0.68	0.95		0.86	1.35	0.81
Al_2O_3	9.98	13.8		14.61	13.99	12.97
Fe_2O_3	0.92	0.86		0.88	1.07	0.81
FeO	9.18	8.62	11.21	8.75	10.67	8.05
MnO	0.17	0.16	0.10	0.16	0.2	0.19
MgO	20.00	10.6	47.33	9.37	6.61	8.66
CaO	8.6	11.9	0.3	11.87	9.31	14.81
Na ₂ O	1.12	1.6		1.7	3.75	1.2
K ₂ O	0.09	0.07		0.13	0.56	0.03
P_2O_5	0.05			0.06	0.1	0.06
Cr ₂ O ₃	0.27	0.08		0.05	0.01	0.08
NiO	0.11		0.46	0.02	0.01	0.03
Mg/(Mg+Fe)	0.80	0.69	0.88	0.66	0.52	
La/Sm	0.9		2.00	0.84	0.95	$0.66 \\ 1.07$
Mg/(Mg ₊ Fe) in a	tomic ratio; Fe ₂ O	$_3 = 0.1 \text{ FeO}$				2.07

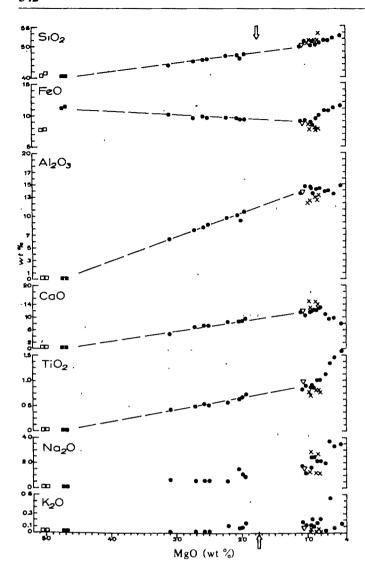


Fig. 1 MgO-variation diagram of Curaçaoan picrites and basalts. •, Picrites, ohvine-phyric tholentes and plagioclase-pyroxene-phyric tholentes; \times , variolitic basalts; \blacksquare , olivine phenocrysts of picrites (Fo_{86 5-87 4}); \square , olivine megacrysts (Fo₉₀ and Fo_{91 4}); \triangle , glass of picrite 79KV615 Arrows indicates MgO percentage of calculated parent liquid using a K_D (ref. 15) of 0.3 and Fe₂O₃ = 0.1

crysts up to 5 mm long and with a composition up to Fo 91.4 (Table 2, Fig. 1) in three of the picritic flows brings us nearer to a conclusion about the composition of the parent. Assuming that they represent early formed, non-equilibrated phenocrysts, the composition of the melt in which they formed can be calculated using Roeder and Emslie's K_D of 0.3 and the MgO variation diagram (Fig. 1). This melt (Table 2) has about 17.5% MgO. A slightly higher K_D would give a parent within the range of the pillowed picrites (MgO 19-22%); the use of an oxidation ratio lower than the value assumed for the picrites (0.1) has the same effect: taking total Fe as FeO gives a parent with 19.9% MgO.

Accumulation of olivine is evident in the picrites with a MgO content of 25-31 wt%. These olivines are rounded and partly resorbed. In the most Mg-rich flows olivine forms up to 60 vol.%.

The control line of the MgO variation diagram (Fig. 1) changes in slope between 8 and 9 wt% MgO, indicating that fractionation of plagioclase and pyroxene defines the composition of the more evolved tholeiites. The variolitic basalts, with a MgO wt% similar to that of the olivine-phyric tholeiites, systematically plot outside the fractionation trend of most of the volcanics of the Curação Lava Formation.

The chondrite-normalized REE patterns (Fig. 2) of picrites, olivine-phyric tholeiites and plagioclase-pyroxene phyric tholeiites are consistent with consanguinity of the suite. They are basically flat (T-type MORB; La/Sm_N = 0.8-1.0). From the MgO/Sm ratio the REE content of the parent can be estimated to be about 6 times chondritic. Assuming that the mantle source is twice chondritic^{1,16}, the parent magma would represent about 30% batch partial melting. This amount of melting would leave a residue of olivine and orthopyroxene only. Although residual rocks of the Curaçaoan succession are not known, a model calculation is given in Table 2, using the olivine megacrysts (Fo_{91.4}) of sample 79Be148, and an orthopyroxene of Green et al.¹. A small amount (0.5%) of a chrome-rich spinel from sample 79KV615 is added to the residue, mainly to raise the Cr₂O₃-content of the source to acceptable values. The model calculations are furthermore constrained by: (1) composition of the residue, that must fall within the field of that of natural, mantle derived periodites¹⁷; (2) 3-4 wt% CaO and 4-5 wt% Al₂O₃ in the source composition, that is compositions similar to previous estimates of upper mantle pyrolite18. The first constraint, in combination with 30% partial melt, mainly defines the proportions of the residual phases as approximately 80% olivine and 20% orthopyroxene. A much higher amount of orthopyroxene would give a residue too high in SiO2 and too low in MgO. The second contraint necessitates the selection of an Al₂O₃-rich orthopyroxene. The residual orthopyroxene of

Table 2 Parental liquid of Curaçaoan basalts, and model calculation of the source

,	Parental liquid, recalculated at 100%	Olivine- megacryst Fo _{91 4} 79Be148	Orthopyroxene*	Spinel 79KV615	Residue $F = 0.3$ 78% ol. 21 5% opx 0.5% spinel	Source	Source of DSDP3-18
SiO ₂	48.33	40.7	54.8		43.53	45.00	45.00
T_1O_2	0.74			0.49	•	0.22	0 17
Al_2O_3	11.35		5.5	19.90	1.28	4.35	4.4
Fe_2O_3	0.94						
FeO	9.35	7.8	4.9	18.2	7.22	8.13†	7.6
MnO	0.17	0.12			0.09	0.11	0.11
MgO	17.70	50.55	32.0	14.3	46.38	37.61	38.8
CaO	9.57	0.2	2.8		-0.76	3.46	3.4
Na_2O	1.34					0.41	0.4
K ₂ O	0.11					0.03	0.003
P_2O_5	0.06		•			0.02	
Cr ₂ O ₃	0.25	0.1	1 2	45.7	0.56	0.47	0.45
NiO	0.09	0.38	0.05	0.19	0.31	0.25	0.26

^{*} From ref. 1.

[†] FeO total.

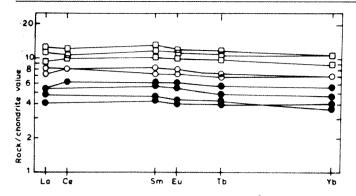


Fig. 2 Chondrite-normalized REE-patterns of representative basalts of the Curação Lava Formation. O, Picrites; O, olivinephyric tholeiites; \square , plagioclase-pyroxene-phyric tholeiites. MgO-content of the basalts are, from base to top: 27.5; 25.1; 21.0; 20.7; 11.0; 10.3; 8.3; 6.0; 4.9.

Green et al.¹ best fits our needs, and gives a source (Table 2), which is in good agreement with that of earlier calculations^{1,18}. The main difference with the source of DSDP 3-18 (ref. 1) is the much higher K₂O-content, reflecting the transitional nature of the Curaçaoan basalts.

The common occurrence of picritic flows in the lower part of the Curação Lava Formation suggests that, at this stage of development of the volcanic unit, magma reservoirs are small. They enlarge as the volcanic pile builds up, and the height of the magma column between reservoir and surface becomes such that the dense picritic liquid can no longer extrude. Fractionation of olivine, and, at a later stage, of pyroxene and plagioclase in these reservoirs, provide the less dense tholeiites of the upper half of the formation. These considerations suggest that the Curação Lava Formation was formed because of the initiation of a new system, rather than being a fragment of oceanic crust fed by a steady-state magma chamber. The new system is not the opening of the Caribbean, as this occurs much earlier¹⁹. Considering the close association of the unit in space and time with volcanic sequences of island-arc origin (late Albian to Coniacian on the neighbouring islands of Aruba and Bonaire²⁰), we consider the unit to be the base of an island-arc succession formed either during the initiation of subduction²¹, or because of lengthening of an existing arc at a trench-trench-ridge triple junction. These models will be discussed more fully elsewhere.

Fieldwork in Curação was made possible through grants WR 75-211, 212 and 213 of the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). We thank M. de Bruin and co-workers, I. S. Oen, H. J. MacGillavry and J. Grocott for help, and ZWO-WACOM for financial and personal support.

Received 16 November 1981; accepted 27 January 1982.

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Seabed-wave resonance and sand bar growth

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The interaction of surface water waves with undulating sea-bed topography is of fundamental importance to coastal engineers and sedimentologists. For example, wave reflection from submerged bars on beaches may provide a mechanism for protecting the beach from further wave attack. Additionally the interaction of surface water waves with other tidally or wave generated bedforms, such as sandwaves on offshore banks, may modify wave climates on adjacent coastlines. While there have been numerous studies of the interaction of sea waves with engineering structures, such as breakwaters, piers, and jetties, there have been comparatively few investigations of wave interactions with naturally occurring bedforms. Predictions have recently been made of the amount of wave energy reflected as a result of resonant interactions between surface water waves and undulating sea-bed topography but with no supporting experimental proof. I describe here preliminary results from what are believed to be the first published laboratory measurements of resonant interactions between surface water waves and submerged bars which show that significant and large amounts of wave energy may be reflected and that these reflections are brought about by resonant interactions between surface-water waves and the bedforms. In particular, at resonance, incident surface water wavelengths are approximately twice the bedform wavelengths. These results have implications not only in terms of wave reflection from naturally occurring bedforms, say bars on beaches, but also for sediment transport processes in general.

Davies^{1,2} has used linear perturbation theory to show that to

a first approximation, wave reflection from a finite number of submerged sinusoidal bars, having small amplitude and on an otherwise plane bed, is given by

$$K_{r} = \frac{a_{r}}{a_{i}}$$

$$= \frac{2bk}{\{2kh + \sinh(2kh)\}} \left(\frac{2k}{l}\right) \frac{\left|\sin\left(\frac{2k}{l}m\pi\right)\right|}{\left(\frac{2k}{l}\right)^{2} - 1}$$
(1)

where K_r is the wave reflection coefficient, a_r and a_i are the reflected and incident wave amplitudes respectively, well away from the region of bedforms, b is the bar amplitude, h is the water depth, m is the number of bars and k and l are the free surface and bar wavenumbers. Here $k = 2\pi/L$ and $l = 2\pi/L_b$, where L and L_b are surface and bar wavelengths respectively. In the theory it was assumed that the wave crests were parallel to the bars and that the flow was non-separating. Additionally, certain constraints were imposed on the result (equation (1)) which included the following: $a_i k$, $a_i h$, $a_i / k^2 h^3$, bl, b/hand $bk \ll 1$. Furthermore, unless the solution is corrected for wave attenuation across the bars (see later) it is required that $|a_r/a_i| \ll 1$.

Following Davies¹, the result in equation (1) may be considered in two parts the second of which we shall denote as

$$H\left(\frac{2k}{l}\right) = \left(\frac{2k}{l}\right) \frac{\left|\sin\left(\frac{2k}{l}m\pi\right)\right|}{\left(\frac{2k}{l}\right)^2 - 1} \tag{2}$$

Equation (2) illustrates that for a given number of bars (m),

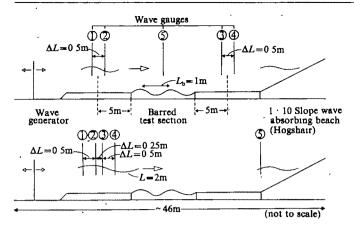


Fig. 1 Positions of gauges, in relation to barred test section and wave absorbing beach, for two main types of measurements. Typical values of the wave gauge spacing ΔL are also shown.

the wave reflection coefficient is oscillatory in 2k/l, that is the quotient of twice the surface wavenumber and the bed wavenumber. The reflection coefficient is also resonant in the region $2k/l \approx 1$ and, at 2k/l = 1 itself, $H(2k/l) = m\pi/2$ which suggests that peak reflection coefficients are linearly dependent on the number of bars present.

To test Davies'^{1.2} theoretical predictions and in particular equations (1) and (2), detailed measurements of wave reflection from submerged bars were carried out using the $45.72 \times 0.91 \times 0.91$ m wave tank facility at the Coastal Engineering Research Center, Virginia. A 10-m long test section consisting of 10×1 m wavelength, 0.05-m amplitude sinusoidal bars was constructed in the tank and set in a false bottom. The barred test section was situated approximately midway between a hydraulically driven piston type wave generator, at one end of the tank, and a 1:10 slope wave absorbing beach at the other. Water surface elevations were measured using standard parallel-wire resistance type wave gauges and wave reflection coefficients determined using the method of Goda and Suzuki³.

Two pairs of gauges and a single gauge were used to make two types of measurement of which some preliminary results are shown here; first incident and transmitted wave conditions were measured with one gauge pair 5 m on the up-wave side of the bars and the second gauge pair 5 m on the down-wave side. The remaining gauge was positioned midway along the test section. The up-wave gauge pair thus gave information on wave reflection from the bars while the second gauge pair provided data on the transmitted wave heights and the amount of wave energy reflected from the beach. In the second type of measurement two pairs of gauges were moved along the tank

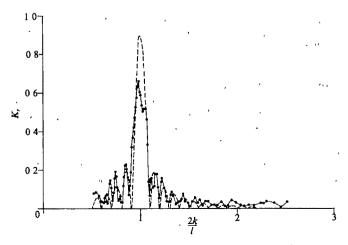


Fig. 2 Comparisons of measured (\bullet) and predicted values (---) of the reflection coefficient, K_r , as a function of the wavenumber ratio 2k/l, for b/h = 0.16 and m = 10 bars.

in such a way as to give surface elevation data every 0.25 m and to determine how wave reflection varied throughout the tank, first from the barred test section and finally from the beach. The remaining gauge was positioned at the end of the tank at the foot of the beach. These experimental arrangements are illustrated in Fig. 1.

With the bar wavelength, L_b , fixed at 1 m, incident surface water wavelengths were varied over a range giving $0.5 \le 2k/l \le 2.5$, by varying the wave period in steps of 0.01 s. For the results shown here, water depths were varied to give bar amplitude—water depth ratios, b/h, in the range $0.08 \le b/h \le 0.16$. Thus, good resolution, in non-dimensional wavenumber space 2k/l, of the order of 0.01, enabled detailed investigations to be made of the oscillatory nature of the wave reflection coefficient and of the resonant interaction peaks. These tests were carried out using small amplitude monochromatic waves only and with wave steepness values which complied with the scaling laws given by Davies and shown previously.

For surface water wavelengths approximately twice the bar wavelength, strong resonant interactions were observed leading

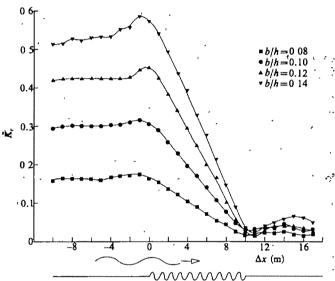


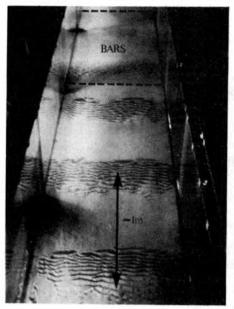
Fig. 3 The reflection coefficient, K_r , as a function of distance along the wave tank, Δx , and different ratios of bar amplitude to water depth, b/h, for m=10 bars. Note that the origin is taken as the position of zero elevation of the first bar and that $K_r \to K_r$ for $-\Delta x/h > 10$.

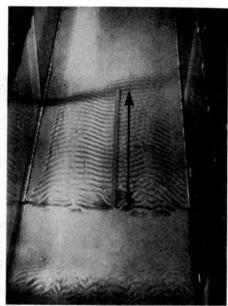
to large reflection coefficients (in some cases as large as $K_r = 0.8$) and dramatic standing or partial standing wave patterns on the up-wave side of the bars. On the down-wave side of the bars the standing wave pattern gave way to progressive waves leaving the test section and travelling towards the wave absorbing beach.

Figure 2 shows the variation of wave reflection coefficient, K_r , with the wavenumber ratio 2k/l for 10 bars and a bar amplitude-water depth ratio of b/h = 0.16 (h = 31.3 cm). Also shown are the first-order predictions from Davies^{1,2}, uncorrected for the effects of wave attenuation as the incident waves propagate over and are reflected by the bars. If the theoretical results are corrected by the method proposed by Davies¹, to relax the condition $|a_r/a_i| \ll 1$ and to give a more realistic result allowing for wave attenuation, the predicted peak value of K_r at $2k/l \approx 1$ is $K_r = 0.75$, which is in quite close agreement with the measured value.

A striking feature of these results is the large resonant interaction peak at $2k/l \approx 1$, with peak reflection coefficients of ~ 0.65 , and the oscillatory nature of K_r in respect of 2k/l. Figure 3 shows the results from measurements of the reflection coefficient at resonance, at different positions, Δx , along the tank and throughout the barred test section. Note that this is a modified reflection coefficient K_r , such that $K_r \to K_r$ for $-\Delta x/h > 10$. Measurements are shown for bar amplitude—water

Fig. 4 Left, ripple patches with a 1-m spacing formed beneath a partial standing wave on the up-wave side of 2×1 m wavelength bars. The bar amplitude is 5 cm and for these observations the water depth was 15.6 cm and the reflection coefficient was $K_r \approx 0.34$. The largest ripples were ~1.5 cm high with wavelengths of about 5.5 cm. Right, a continuous sheet of ripples formed down-wave of the reflecting bar system. Ripple heights were of the order of 1.0-cm with wavelengths of about 4 cm.





depth ratios in the range $0.08 \le b/h \le 0.14$ that is $35.7 \le h \le$ 62.5 cm. The resonant wave periods varied from 1.17 to 1.28s. dependent on actual water depth, and were usually within 0.02s of the predicted values. These results indicate that on the up-wave side of the bars the reflection coefficient, $K_r \rightarrow K_r$, is more or less constant and rises to a peak value within a few water depths of the bars before falling, linearly throughout the test section, to values of order 0.05 or less, which is the reflection from the beach alone. While only preliminary measurements for 10 bars are shown here, later measurements on 4, 2 and 1 bars have shown similar trends to those given in Figs 2 and 3. These and other observations are to be reported more fully elsewhere.

Davies^{1,2} has suggested that as a result of the partial standing wave which forms up-wave from a reflecting bar system, the pattern of wave orbital motions near the bed may lead to areas of preferential erosion and deposition of sediment. Potentially, at least, this provides a mechanism for bars to grow in the up-wave direction.

To confirm this result fine sand of about 235-µm mean diameter was sprinkled in a thin uniform layer throughout the barred test section (with 2 bars only) and for about 2-3 m on either side of it. Waves were started from rest and the wave amplitude increased until sediment motion was initiated. Sediment movement was then observed for a resonant wave reflection condition $(K_r \approx 0.34)$ and the evolution of ripple patches recorded on the up-wave and down-wave side of the bars. Figure 4 shows results from these experiments. The important features are the formation of ripple patches with a 1-m spacing on the up-wave side of the bars (Fig. 4a) and the appearance of a more or less continuous sheet of ripples on the down-wave side (Fig. 4b). Both these features are associated with a 2-m surface wavelength. In Fig. 4a erosion and ripple formation was observed to occur beneath the nodes of surface elevation of the partial standing wave. With increasing time ripple heights were observed to grow on the up-wave side of each patch in such a way as to bring about an accumulation of material approximately midway between node and antinode and roughly in the position where bar crest formation would be expected to occur. These results confirm that a potential bar-growth mechanism occurs up-wave of the bars (Fig. 4a), but not on the down-wave side (Fig. 4b), and while these results cannot be extended unambiguously to the case of naturally occurring sand bars on an erodible bed, there is strong evidence to suggest that such a mechanism may occur in practice. Note that Nielsen4 also found evidence of bar growth beneath standing waves (not generated by bars) and in particular that fine sand (80 µm), moving mainly in suspension, accumulated

directly under the antinodes. The results described here are for coarser material moving principally as bedload and the resulting distribution of sediment is governed more by velocities near the bed and less by the convection cells described by Nielsen.

I thank the Commander and Director of the US Army, Corps of Engineers, Coastal Engineering Research Center, Fort Belvoir, Virginia, USA, for experimental facilities, and also Dr A. G. Davies for valuable discussion.

Received 7 December 1981: accepted 11 February 1982

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Downcore variation in sediment organic nitrogen

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An historical record of natural (pre-cultural) and anthropogenic (influenced by man's activities) chemical inputs to the environment is contained in accumulating sediment deposits and is commonly expressed as a depositional flux1. This flux is traditionally calculated as the product of the sediment accumulation rate and the concentration of a substance in the sediment1. Previous work has quantified depositional fluxes of metals and nutrients by assuming that the vertical distribution of these substances in sediments is unaffected by post-depositional migration, chemical reaction or porosity variations²⁻¹⁰. We present here a procedure for calculating the historical depositional flux (loading record) of a substance which undergoes post-depositional chemical reaction in a compacting sediment column. The method is demonstrated by calculating the loading record of organic nitrogen at one locality in Lake Erie.

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Organic nitrogen was selected for study because its vertical distribution in Lake Erie sediments (41°42.0′ N; 82°18.8′ W) depends solely on the depositional flux of particulate organic nitrogen and the post-depositional rate of microbial decomposition of organic nitrogen; nitrogen does not participate in mineral equilibrium reactions in Lake Erie sediments. Microbial degradation of particulate organic nitrogen compounds converts their contained nitrogen directly to ammonium¹¹. We have assumed that the destruction of particulate organic nitrogen may be quantified by measuring net ammonium production. Because some of the produced ammonium may be converted to other nitrogen species, the assumed rate of decomposition of particulate organic nitrogen is a minimum. As a result, the calculated loading record for organic nitrogen represents the minimum organic nitrogen loading record at this locality.

The rate of nitrogen loss from sediment solids (or gain by interstitial water) is proportional to the concentration of particulate organic nitrogen present¹¹⁻¹³. This is illustrated with data from the Lake Erie study site in Fig. 1. At any depth z (measured positively downwards) this may be expressed as

$$R(z) = -k(z)N_{P}(z) \tag{1}$$

where $N_{\rm P}(z)$ is the concentration of particulate organic nitrogen at depth z (mol N per mass sediment particles), k(z) is the apparent rate 'constant' (time⁻¹) at depth z, and R(z) is the rate of ammonium production at depth z. Note that k(z) is not a constant. It is a function of the nature of the particulate organic nitrogen undergoing decay, and, consequently, depth in the sediment. In addition, k(z) represents the resultant of many chemical reactions. Nevertheless, it is convenient to think of k(z) as an apparent integrated rate constant at depth z. It is implicitly assumed that all particulate organic nitrogen present can be utilized by bacteria. The continuity equation describing the change in nitrogen content in a mass of sediment particles travelling downward from the sediment—water interface is

$$\frac{\mathrm{d}N_{\mathrm{P}}}{\mathrm{d}t}(z,t) = \frac{\partial N_{\mathrm{P}}}{\partial t} + \omega(z)\frac{\partial N_{\mathrm{P}}}{\partial z} = R(z) \tag{2}$$

where $\omega(z)$ is the downward velocity of sediment particles at depth z due to sedimentation. ω is given by

$$\omega(z) = q/\rho(z) \tag{3}$$

where q (assumed constant) is the mass flux of sediment particles to the sediment-water interface (mass/area/time) and $\rho(z)$ is the mass of sediment particles contained in a volume of sediment at depth z:

$$\rho(z) = \rho_s(1 - \phi(z)) \tag{4}$$

where ρ_* is the density of sediment particles and $\phi(z)$ is the ratio of void volume to total sediment volume at depth z. Assuming a constant flux of particles to the sediment—water

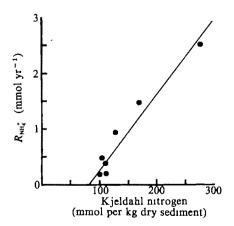


Fig. 1 Rate of ammonium production versus Kjeldahl nitrogen for sediments from the Lake Erie locality. Data from ref. 13.

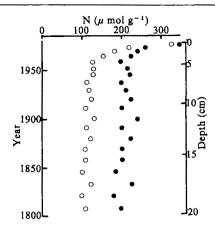


Fig. 2 Present-day particulate organic nitrogen concentration (N_P, \bigcirc) and calculated particulate organic nitrogen concentration at the time of deposition (N_{PO}, \bullet) plotted against depth and time of deposition at the coring locality.

interface and steady-state compaction, the solution to equations (1)-(4) is

$$N_{\rm P}(z,t) = N_{\rm po} \exp\left[-\frac{1}{q} \int_0^z k(z) \rho(z) \,\mathrm{d}z\right] \tag{5}$$

where t =present time and

$$N_{\rm po} = N_{\rm P} \left(t - \frac{1}{q} \int_0^z \rho(z) \, \mathrm{d}z \right)$$

 $N_{\rm po}$ is the concentration of particulate organic nitrogen at the time of deposition. The historical record of $N_{\rm po}$ can be calculated using equations (1) and (5). Measured values of $N_{\rm P}(z)$ (as total Kjeldahl nitrogen), R(z) (as ammonium production)¹³, $\phi(z)$ (calculated from water content and $\rho_{\rm p}$), and q (by ²¹⁰Pb; q=0.077 g cm⁻² yr⁻¹) (J. A. Robbins, personal communication) were used in the calculation. The values of R(z) represent net rates for ammonium production. Conversion of produced ammonium to other nitrogen species or adsorption onto sediment particles¹⁴ were not accounted for in the production rate experiments. The results of this calculation are shown in Fig. 2 along with the measured present day concentration of particulate organic nitrogen, $N_{\rm P}$.

The data presented in Fig. 2 indicate that the loading of particulate organic nitrogen to sediments at the coring locality has increased since ~1950. Scatter in the data preclude a detailed examination of particulate organic nitrogen flux before this date. The results are in reasonable temporal agreement with estimates of phosphorus input to Lake Erie¹⁵. In the early nineteenth century (1820-60), the flux of particulate organic nitrogen qN_{po} was ~0.165 mol m⁻² yr⁻¹. In the recent past (1974-77), the flux of particulate organic nitrogen was ~ 0.285 mol m⁻² yr⁻¹. This gives a current ratio of anthropogenic to natural nitrogen loading of ~0.73. This result is substantially less than estimates of this ratio made by Kemp et al.5. These workers calculated the average present ratio of anthropogenic to natural nitrogen loading to Lake Erie sediments as ~1.79 ± 0.78. They did not consider decomposition of organic matter containing nitrogen. In fact, the values of N_{ro} calculated here are minima. The rates of ammonium production used do not include any conversion of ammonium to nitrate or nitrogen gas. Further, loss of particulate organic nitrogen due to oxygen and nitrate reduction in the uppermost portion of the sediment column is completely neglected¹³. Inclusion of these reactions would increase N_{po} and lower the anthropogenic to nitrogen loading ratio. The omission of the decomposition of particulate organic nitrogen in the calculation of organic nitrogen loading to sediments will increase the apparent ratio of anthropogenic to natural nitrogen loading. For example, the application of the methodology used by Kemp et al.5 to our organic nitrogen data yields a current anthropogenic to natural nitrogen loading ratio of 1.99. The neglect of organic decomposition in estimates of

man's effect on the lake's nitrogen budget increases the apparent anthropogenic contribution by ~ 2.7 times.

The method outlined above for incorporating decomposition should be tested at other sites and, with appropriate modifications, applied to other reactive components such as phosphorus and carbon. This approach could produce significantly different nutrient budgets in many environments and may lead to a reevaluation of some management criteria.

This is contribution no. 141 Department of Geological Sciences, Case Western Reserve University. Financial support was provided by EPA contract R8057160 (D. Dolan, project manager) and NOAA contract NA80RAD00036.

Received 24 September 1981; accepted 28 January 1982.

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Eocene to Oligocene benthic foraminiferal isotopic record in the Bay of Biscay

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We present here oxygen and carbon isotopic records of Eocene to Oligocene benthic foraminifera from two Bay of Biscay Deep Sea Drilling Project (DSDP) sites (119 and 401). δ^{18} O of benthic foraminifera increases 1.9% from a middle Eocene minimum (Zones P10-P11) to an earliest Oligocene maximum (Zone NP21). Approximately 1.4% of the increase in benthic foraminiferal δ^{18} O occurs during the late Eocene to earliest Oligocene (Zones P15/16-NP21). Previous results from other North Atlantic DSDP sites (400A and 398) have significantly lower δ^{18} O values of benthic foraminifera, some by as much as 2% (refs 1-3). We believe that these differences result from diagenetic alteration of the sediments in the deeper-buried Sites 400A and 398.

Previous reports of Pacific and Southern Ocean DSDP sites noted that a 1.0-2.0% enrichment of ¹⁸O occurs from the middle Eocene to early Oligocene in both planktonic and benthic for a minifera and that $\sim 1\%$ of this change occurs near the Eocene-Oligocene boundary⁴⁻⁹. Eocene to Oligocene δ^{18} O records from three DSDP locations depart from this general Pacific-Southern Ocean trend: (1) in the Philippine Sea (Site 292) the ¹⁸O enrichment occurs only in benthic foraminifera⁹; (2) in the South Atlantic (Site 357), an isotopic enrichment occurs between the middle and late Eocene¹⁰; and (3) in the North Atlantic (Site 398) the magnitude of the isotopic increase is twice that noted in the Pacific and Southern Oceans and the enrichment occurs near the middle/late Eocene boundary^{1,2}

The Eocene to Oligocene oxygen isotopic enrichment in the North Atlantic is poorly documented. Strong diagenetic effects on isotopic composition were noted in sites drilled in the Bay of Biscay (Site 400A) (refs 1, 3, 11) and off Portugal (Site 398) (refs 1, 2, 12, 13). Eocene benthic foraminiferal δ^{18} O values are low and calculated palaeotemperatures are correspondingly high (~17°C)^{1,2} relative to Pacific, Southern Ocean⁴⁻⁹, and South Atlantic¹⁰ δ^{18} O records. Such low δ^{18} O values and high inferred palaeotemperatures may result from diagenetic effects. It is necessary to distinguish between altered isotopic signals and original unaltered signals to determine abyssal palaeocirculation patterns.

We analysed the oxygen and carbon isotopic composition of early-middle Eocene to Oligocene benthic foraminifera at two North Atlantic DSDP sites (119 and 401). We chose these sites for the shallow burial depth of their Eocene sediments (~100-400 m) versus Sites 400A and 398 (~550-600 m). Late Eocene sediments are missing from Site 119. However, drilling at nearby Site 401 recovered a relatively complete Eocene section, but no Oligocene section¹⁴. Site 119 was drilled in 4,447 m of water on Cantabria Seamount¹⁵. Using the backtracking

Table 1 Oxygen and carbon isotopic data for Sites 119 and 401

Sample	Zonal age (Myr)	Taxa	$\delta^{18}{ m O}$	δ^{13} C
Site 119				
12-CC	NP25 (24.0)	Cibicidoides	1.15	0.39
13-2 146-149 cm		Cibicidoides	1.40	0.30
13-CC	NP25 (26.3)*	Cibicidoides	1.54	0.53
14-CC	P21, NP24 (28.7)	Cibicidoides	1.36	-0.03
15-CC	P20/21, NP24 (31.0)*	Cibicidoides	1.61 1.52	-0.01 -0.33
	(51.0)	Gyroidinoides	1.24	-0.47
		<i>O</i> ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	1.21	-0.46
			1.72	-0.68
		Catapsydrax	0.81	-0.59
16-2 119-121 cm	NP23 (31.6)	Cibicidoides	1.27	0.06
16-CC	NP23, P20/21	Cibicidoides	1.81	0.56
	(32.3)*	5 - 6 st	1.78	0.42
	,	Gyroidinoides	2.26	-0.06
		and the second	2.17	-0.23
		Catapsydrax	1.12	0.52
17-5 146-149 cm and 17-CC	NP22	Catapsydrax	0.84	0.71
17-CC	NP22 (34.7)	Cibicidoides	1.59	0.75
	,	Gyroidinoides	2.05	0.53
18-2 144-147 cm	NP21/22 (35.7)	Cibicidoides	1.35	0.47
18-CC	NP21 (37.0)	Cibicidoides	1.96	1.28
			2.02	1.27
		Gyroidinoides	2.56	0.76
			2.34	0.46
19-CC	Lower NP15 (47.0)	Cibicidoides	0.14	0.38
20-3 top	NP15 (47.4)	Cibicidoides	0.42	0.44
20-CC	NP15 (47.6)	Cibicidoides	0.43	0.53
		Gyroidinoides	0.79	0.05
21-CC	NP13 (49.3)	Cibicidoides	0.73	0.51
		Gyroidinoides	0.62	0.03
Site 401				
2-1 8-10 cm	G. cerrolazulensis			0.00
	(37.5)	Cibicidoides	1.26	0.89
3-1 15-21 cm	P15/16 (38.9)	Cibicidoides	0.64	0.67
4-CC	P14 (41.0)	Cibicidoides	0.86	0.55
5-3 93-97 cm	P13 (42.8)	Cibicidoides	0.22	0.14
6-3 76-80 cm	P12 (43.9)	Cibicidoides	0.62	0.53
7-3 76-80 cm	P12 (45.3)	Cibicidoides	0.20	0.73
8-3 44-48 cm	P11 (46.6)	Cibicidoides	0.43	0.65 0.63
9-3 96-100 cm 10-3 98-102 cm	P10 (47.7) P10 (49.0)	Cibicidoides Cibicidoides	0.45	0.63
10-3 98-102 cm	F10 (49.0)	Civiciaoiaes	0.42	0.49

Nannoplankton zonations for Site 119 after ref. 20 and M.-P. Aubry, personal communication. Foraminiferal zonations for Site 119 after ref. 19 and K.G.M. work in preparation and for Site 401 after ref. 14.

Discrepancies resulting from differences between zonal age and interpolated ages (indicated in parentheses in Myr) obtained by assuming constant sedimentation rate.

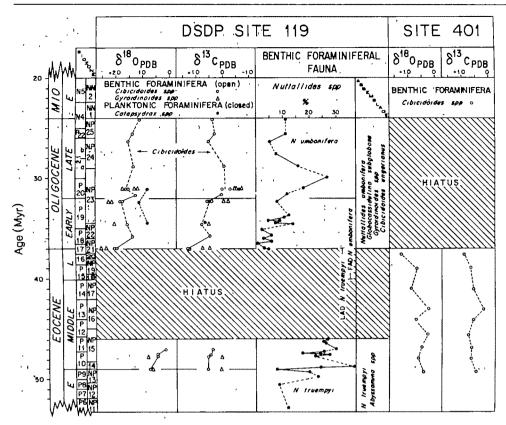


Fig. 1 Age versus foraminiferal isotopic composition for Sites 119 and 401 and faunal composition for Site 119. Last appearance datum (LAD) of N. truempyi and first appearance datum (FAD) of N. umbonifera after ref. 39.

method of Berger and Winterer¹⁶, we calculate that Site 119 was deeper than 3,000 m throughout the Eocene and Oligocene. Site 401 was drilled in the Bay of Biscay on the Armorican margin in 2,495 m of water¹⁷. Palaeobathymetric estimates place Site 401 near its present depth throughout the early Tertiary¹⁸. Biostratigraphic age control for the sites is taken from the *Initial Reports of the DSDP* Legs 12 (refs 15, 19–21) and 48 (refs 14, 17, 22); a biostratigraphic review and detailed palaeobathymetric estimates will be presented elsewhere (K.G.M., work in preparation).

Isotopic analyses were performed on mixed species of the genus *Cibicidoides*. Interregional isotopic variations of this taxon in Holocene core tops reflect the distribution of temperature, δ^{18} O of seawater, and δ^{13} C of Σ CO₂ in the modern

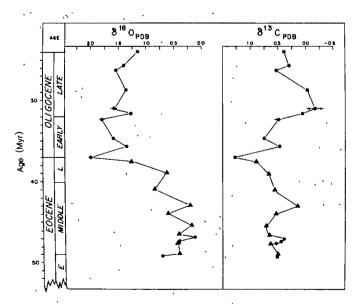


Fig. 2 Combined oxygen and carbon isotopic record obtained for Cibicidoides from Sites 119 (and 401 (). Where more than one value was obtained per sample, the mean value was plotted and the range indicated with arrows.

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ocean²³⁻²⁶. Although Cibicidoides species apparently secrete their tests lower in δ^{18} O than values calculated from the Epstein et al.²⁷ palaeotemperature equation, the offsets seem to be constant²³⁻²⁶. In addition, we analysed paired samples of Gyroidinoides spp. and Cibicidoides spp. from Site 119; Gyroidinoides were $\sim 0.3\%$ enriched in ¹⁸O relative to Cibicidoides. This offset is similar to differences observed between these taxa in studies of Holocene core—top sediments²⁶.

At Site 119, δ^{18} O values increase from $\sim 0.5\%$ in the early-middle Eocene (nannoplankton Zones NP13 to NP15) to $\sim 2.0\%$ in the earliest Oligocene (Zone NP21) (Fig. 1); δ^{13} C values correspondingly increase from ~ 0.4 to $\sim 1.3\%$ from the middle Eocene to earliest Oligocene. Unfortunately a 9-Myr hiatus precludes determination of the exact timing and nature of this isotopic change between the middle Eocene and earliest Oligocene in Site 119. In the Oligocene, δ^{18} O values decrease from ~ 2.0 to $\sim 1.1\%$. δ^{13} C values decrease to a minimum of $\sim 0.0\%$ in the middle Oligocene (Zones P20–P21), then increase again to $\sim 0.5\%$. At Site 401 (Fig. 1), δ^{18} O values are $\sim 0.5\%$ in the early-middle Eocene, similar to observed values in Site 119. δ^{18} O values increase $\sim 0.4\%$ in the late middle Eocene (Zone P14). A δ^{18} O increase of 0.6% occurs within the late Eocene (Zones P15/16–P17).

Combining the isotopic record of both sites (Fig. 2) suggests that a δ^{18} O increase of ~1.4% occurs from the late Eocene to earliest Oligocene in the North Atlantic. This change occurred within an interval that may be <1 Myr (that is within Zone NP21) or as much as 4 Myr (between Zones P15 and NP21; see biostratigraphic zonations Table 1). There are pitfalls in combining sites of different palaeodepths to obtain a complete record; however, δ^{18} O values obtained for Sites 119 and 401 are similar in the overlapping section (middle Eocene). The modern temperature difference between these water depths in the Bay of Biscay is <1 °C (ref. 28), equivalent to an oxygen isotopic difference of <0.25%. These reasons justify combining the δ^{18} O records of Sites 119 and 401. Although the middle to late Eocene record may be found entirely within Site 401, the nature and timing of the δ^{18} O shift near the Eocene-Oligocene boundary involves comparing records from two different sites, and should be considered as preliminary. The Eocene-Oligocene interval in the North Atlantic is usually

discontinuous^{29,30}, and a complete Eocene to Oligocene isotopic record from one site is not available at present.

The δ^{18} O records of Sites 119 and 401 apparently correlate with isotopic records from the Pacific and Southern Oceans⁴⁻⁸ where an ¹⁸O enrichment occurs in the late Eocene to early Oligocene. In detail, the curves may not correlate. Keigwin⁹ showed that the ¹⁸O enrichment occurs in the Pacific above the Eocene-Oligocene boundary, while in our record the change occurs from the late Eocene to earliest Oligocene. However, given the errors of the biostratigraphic age assignments, we consider that the enrichments may be synchronous. Further biostratigraphic studies are needed to determine if the enrichments are diachronous or synchronous.

Although the δ^{18} O values we report are similar to values obtained from sites in the Pacific and Southern Oceans⁴⁻⁹, they are markedly higher than Eocene δ^{18} O values obtained from other sites in the North Atlantic¹⁻³ (Fig. 3). In the Eocene, benthic foraminiferal δ^{18} O in Sites 400A and 398 are as much as 2% lower than in Sites 119 and 401 (Fig. 3). Such implied differences in bottom water temperature (8 °C) between sites of similar palaeodepth (for example, Sites 400A and 119) within the small basin of the Bay of Biscay are oceanographically unreasonable. As the total range of δ^{18} O values for benthic foraminifera within any Recent core-top sample²³⁻²⁶ or Palaeogene sample¹⁰ is <1%, the 2% difference probably cannot result from sampling biases caused by selective dissolution or analysis of mixed benthic foraminiferal assemblages. However, the low values δ^{18} O observed can result from diagentic alteration of the foraminiferal test at high temperatures associated with great burial depth^{2,13}. While simple addition of calcite overgrowths of the test would be sufficient to lower δ^{18} O values, recrystallization of the foraminiferal calcite is necessary to produce both the similar δ^{18} O values of planktonic and benthic foraminifera in the Eocene of Sites 400A and 398 and the high bottom water temperatures (~17 °C) calculated for these sites¹⁻³. At Site 401, δ^{18} O of planktonic foraminifera are substantially lower than benthic foraminifera3, supporting the idea that this site is not substantially affected by diagenetic alteration. Similarly, planktonic foraminifera have substantially lower δ^{18} O composition than benthic foraminifera in the Oligocene of Site 119; due to dissolution, sufficient planktonic

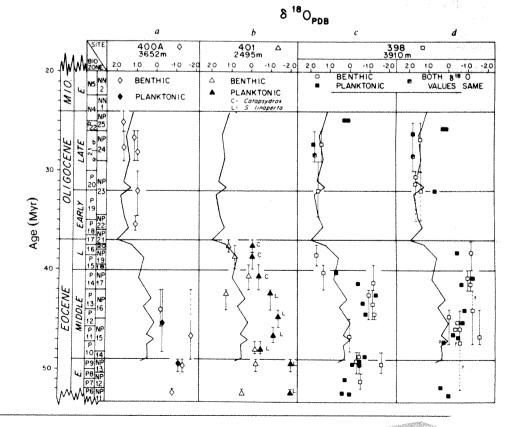
foraminifera were not available for isotopic analyses of the Eocene of Site 119.

Limits can be calculated for the abyssal temperature change in the Bay of Biscay. Shackleton and Kennett⁶ estimated that δ^{18} O of seawater before the formation of the present ice sheets was -1.2% (PDB). Assuming that Cibicidoides precipitates CaCO₃ 0.65% lower than equilibrium²⁶ and that there was no significant glacial ice buildup, the increase in benthic foraminiferal δ^{18} O from Sites 119 and 401 corresponds to a maximum decrease of bottom-water temperatures from 8 to 2 °C between the middle Eocene and early Oligocene. Alternatively, assuming glacial buildup equivalent to the present ice sheets, the increase represents a minimum change in bottom-water temperature from 8 to 6 °C.

The benthic temperature drop in the late Eocene to early Oligocene in the Pacific and Southern Oceans has been attributed to the first formation of bottom waters in the Antarctic region⁷; however, the North Atlantic basins may have had a different source of cold bottom water. Seismic stratigraphical evidence from the northern North Atlantic Ocean indicates that northern sources of vigorously circulating bottom water began in the late Eocene to early Oligocene^{30–32}. Miller and Tucholke³⁰ suggested that this bottom water was of Arctic origin and that it flowed through the Norwegian–Greenland Sea, the Faeroe–Shetland Channel, and possibly through the Denmark Straits into the North Atlantic.

The isotopic changes observed in Site 119 correlate with a change from a benthic foraminiferal assemblage dominated by Nuttallides truempyi to an assemblage dominated by N. umbonifera (Fig. 1); however, the hiatus prevents an exact determination of the timing of this faunal change. Within the Oligocene, N. umbonifera reaches a peak in abundance correlating with a δ^{13} C minimum. As N. umbonifera is negatively correlated with carbonate saturation in the modern ocean³³ and lower δ^{13} C values are often associated with older water masses³⁴⁻³⁶, we speculate that the isotopic and faunal records of Site 119 reflect a change from younger, less corrosive water in the early Oligocene to older, more corrosive bottom water in the middle Oligocene. The change from lower carbon and oxygen values in the Eocene to higher carbon and oxygen values in the earliest Oligocene may reflect a change from older and

Fig. 3 Comparison of previously reported1 Eocene to Oligocene O values for benthic and planktonic foraminifera from North Atlantic DSDP sites. Combined oxygen isotopic curve obtained from Sites 119 and 401 is superimposed on each (solid line). Columns a and b are from Bay of Biscay Sites 400A and 401, respectively. Columns c and d are from Site 398 off Portugal: c represents ages assigned using cal-, d repcareous nannoplankton resents the same data with ages assigned using planktonic foraminifera³⁸. Biostratigraphic ages for Sites 400A and 401 have been assigned using planktonic foraminifera 14 and nannoplank-. Error bars indicate length of biostratigraphic zone(s) to which the sample has been assigned; they have been omitted for clarity from planktonic foraminiferal δ^{18} O values in columns c and d.



warmer Eocene bottom water to colder, younger, more vigorously circulating bottom waters of northern origin in the early Oligocene.

We thank P. E. Belanger, D. A. Johnson, L. D. Keigwin, S. M. Savin, and B. E. Tucholke for critical reviews, T. Poag for technical assistance, F. Heide for drafting, and R. K. Matthews for use of the Benedum Stable Isotope Laboratory of Brown University. Samples were provided by the DSDP. This research was supported by the United States Navy, Office of Naval Research under contract N00014-79-C-0071, a graduate fellowship from Phillips Petroleum and the Woods Hole Oceanographic Institution to K.G.M., a postdoctoral fellowship from the Woods Hole Oceanographic Institution, and NSF grant OCE80-24608 to W.B.C. This is WHOI contribution no. 5008.

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Compound eyes project stripes on the optic tectum in Xenopus

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In the vertebrate nervous system it is commonly found that when two similar nervous structures innervate a third, each innervating structure colonizes distinct areas of the target in a striped manner. Much of our knowledge of the rules of neuronal connectivity derives from the study of the development of the frog visual system, which has the advantages of being simple, and accessible throughout embryonic development. When two separate eyes are made to innervate a single frog optic tectum, the two projections separate into stripes, reminiscent of the ocular dominance stripes of the mammalian visual cortex1. We report here an experiment in which the projection from a single eye, surgically constructed in the embryo out of two matching half-eyes (a 'compound' eye), separates out into stripes. The observation that a single eye can produce a striped projection rules out many hypotheses of how stripes are formed, and provides evidence that the formation of an ordered projection on the optic tectum requires the operation of two separate machanisme

In lower vertebrates, for which much is known of the mechanisms of neural connectivity^{2,3}, the retinotectal projection is completely crossed, and direct binocular innervation occurs by a secondary pathway⁴. Experimental manipulation is therefore needed to induce two retinae to innervate the same target. It is then found that the tectum is divided up between the fibres from the two eyes, in some cases to form eye-specific patches. and in others stripes⁵⁻⁹. In order for stripes to form, there must be some means of distinguishing the fibres from one retina from those from the other; three possible mechanisms come to mind. (1) Fibres carry a record of their side of origin, which enables those from a left eye to be distinguished from those from a right eye. The same effect would be produced if the positional information in the eye were carried by a varying signal such as a biological oscillator¹⁰. The two eyes could therefore be out of phase. (2) Terminal arbors are only stable on their target if neighbouring arbors are from neighbouring ganglion cells. (3) The mechanics of fibre growth are such that the fibres from two separate eyes remain segregated through to their site of termination.

There are various ways of producing double innervation of the visual centres in lower vertebrates, but to determine which of the above theories is correct, a useful technique is surgically to place together in the same eyecup of an embryonic Xenopus, two half-eye rudiments of matching retinal origin (for example, two nasal halves) to create a compound eye11. This results in a retinotectal map in which each of the two half-eyes projects across the entire tectum, superimposed on each other, and with corresponding retinal positions of origin mapping to the same area of tectum. In Xenopus the operation is done before any nerve fibres have grown out of the eye, and there is therefore minimal disruption of the normal development of the visual system; the fibres follow the normal pathway to the brain. This also allows the full development of the projection to be studied, and its topography can be assessed electrophysiologically, so that the relationship between the mechanisms of map-making and stripe formation can be studied.

Compound eyes were made by standard procedures¹¹. One half of the eye rudiment of a stage 29-31 Xenopus embryo was replaced with the opposite half from another embryo of the same age, so that the eye had two nasal halves (NN), two ventral halves (VV) or two temporal halves (TT). The donor half was from the opposite side of the head. Three of the animals also had the other eye removed. The animals were reared for 1-2 months after metamorphosis, then the projections made by the compound eyes were assessed.

The success of the compound eye operations was confirmed by electrophysiological mapping. The retina was then lesioned near the optic nerve head so as to cut most of the fibres from either the host or donor half, and a ~20% solution of horseradish peroxidase (HRP) was then injected over the lesion. After 48 h, the animal was anaesthetized, then perfused and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer. The brain was removed and processed using the method of Adams¹² with the following modifications: incubation was in 1% CoCl₂ for 1 h, then in diaminobenzidine acid solution for 90 min, followed by the addition of $0.3\%~H_2O_2$ (4 ml per 100~ml) at $0~^{\circ}C$ for 20~min. The brains were cleared in methyl salicylate, and examined as whole mounts. A total of 11 animals were examined, of which 9 had both good maps and HRP fills.

Electrophysiological mapping was carried out using standard techniques. In all cases the maps obtained were of the classical compound-eye type^{11,13}. Most of the recording positions produced responses that could be driven by two distinct areas of the visual field. These areas were so arranged that (1) each of the constituent halves of the compound eye projected over the entire tectum in such a way that areas of retina of similar origin projected to the same area of tectum, and (2) the orientation of each map was that predicted if the appropriate half-eye were part of a normal eye.

In all cases the neuropil was labelled discontinuously. The neuropil was fairly superficial, and fibres of passage were seen

at the same depth and deeper than it. In eight of the nine cases the tectum was striped. Typically there were four or five labelled areas. As measured in the cleared preparations, the stripes were of constant width (60-80 µm) and there was a constant interstripe distance of 60-80 µm. The stripes ran rostrocaudally in all cases, and were usually straight, or sometimes slightly wavy. In some cases the stripes coalesced at one extremity of the tectum. This could have been due either to the HRP leaking into the half-eve that it was not intended to fill, or to the mechanism of stripe formation (see below). The actual part of the tectum that was covered depended on the class of compound eye, since the area immediately surrounding the optic nerve head projects to different tectal areas in different classes of compound eye, and it was not possible to fill the chosen half-eye right up to the optic nerve head. Thus in the NN preparations there was no filling of neuropil at the front of the tectum; the midline of the NN eye projected to the rostral tectum. It made no difference to the result whether the host or donor half of the eye was filled.

Optic fibres followed the normal route to the brain, and the manner of their delivery to the tectum was characteristic of the pathways of the various types of compound eye (refs 14, 15 and J.W.F. and R. M. Gaze, in preparation). The only structure that showed striping was the neuropil; the fibres running to the areas of neuropil formed an even sheet over the tectal surface, with no evidence of being divided into separate bands each heading to a specific stripe. No stripes were observed in the optic tract. In addition, fibres of passage were seen between the stripes. In the NN preparations, these fibres ran rostrocaudally, as did the stripes (Fig. 1). In the case of the VVs, however, the fibres ran across the tectum in a rostro-medial to caudo-lateral direction, having all arrived at the tectum by the medial brachium of the tract. The stripes, however, still ran rostrocaudally (Fig. 2). The pretectal nucleus, neuropil of Bellonci and basal optic nucleus, where filled, showed no striping.

In our preparations the fibres from the two half-retinae making up the compound eye travel in a single optic nerve along a normal pathway to the optic tectum. Stripes are visible only in their terminal arborizations. This rules out any mechanism relying on fibres maintaining contact with their original neighbours throughout the optic pathway; nor can stripes be due to the fibres from one eye taking an abnormal pathway, as could be the case in 'three-eyed' frogs⁷. Our experiment also rules out the possibility of stripes being due to an oscillating positional signal.

The two possible mechanisms remaining, therefore, are that either left and right eyes carry specific labels, or that there is a mechanism which stabilizes arbors that are neighbours on the

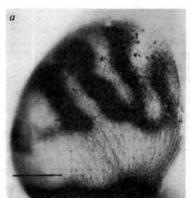




Fig. 1 a, Dorsal view of a whole mount of a tectum innervated by a double nasal eye: medial tectum to the right, caudal to the top. The donor half of the eye was filled with HRP. Stripes run rostrocaudally; the fibres running to them are unstriped. Scale bar, 200 μm. b, Detail from a. Fibres of passage can be seen running between and below the stripes. Scale bar, 100 μm.

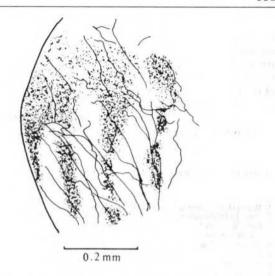


Fig. 2 Camera lucida drawing of a whole mount of a tectum innervated by a double ventral eye. Orientation as in Fig. 1. The stripes again run rostrocaudally, but the fibres to them run from rostro-medial to caudo-lateral tectum.

tectum, and whose retinal ganglion cells are also neighbours. The data of Law and Constantine-Paton⁹ make the first possibility unlikely, but we are testing this by constructing compound eyes in which both halves are from the same side of the head. The second mechanism would result in stripes coalescing in the region to which the retinal midline projects; our results provide some evidence for this.

The neurophysiological and neuroanatomical evidence strongly suggests that the mechanism responsible for the orientation and internal ordering of the retinotectal map in amphibia depends on a set of labels encoding retinal position^{2,3,16}. This allows, for example, fibres from two originally nasal poles of a double nasal eve to project to the same area of tectum. The presence of stripes, however, seems a direct contradiction of this; the two sets of fibres are directed to the same area of tectum only to be separated again. It seems probable, therefore, that the retinotectal map forms in two stages: first the ingrowing optic fibres are guided to terminate on an area of tectum which is determined by the positional labels carried by the retinal ganglion cells from which they came. Once on the tectum, the fibres produce terminal arborizations which interact according to a different set of rules, as discussed above; in doubly innervated tecta this results in the formation of stripes. This is consistent with the suggestion that stripes develop from an initially diffuse projection8. It would certainly require less rearrangement of fibres to produce stripes from a retinotopic map than retinotopy from a striped but disordered one.

In normal development, we believe that the map orientation and a rough degree of retinotopic order are defined by the pathways which fibres follow in growing on to the tectum. Interactions between neighbouring arbors of the type that lead to stripe formation on doubly innervated tecta refine the map and allow it to accommodate to the arrival of new fibres and the growth of the tectum. These two mechanisms correspond to the division suggested by Law and Constantine-Paton⁸.

The stripes in our preparations always ran rostrocaudally. This orientation is clearly not determined by the direction of ingrowth of the optic fibres, as when this is altered (as in the VV animals), the stripes still run rostrocaudally. The most obvious explanation for the direction is that it reflects the mode of growth of the tectum, which grows from its caudal margin¹⁷. Thus, patches of fibres from the two half-eyes formed when the tectum is very small might be expected to extend caudally as the tectum grows. In *Xenopus*, when two optic nerves are induced to innervate a fully grown tectum by regeneration (ref.

6 and D.J.W. and R. M. Gaze, in preparation), the regions of tectum innervated by one eye are not stripes but patches, with no discernible orientation. This may reflect the fact that the tectum is already fully grown and the initial regenerated projection is disorganized¹⁸. It is interesting that the constant width of the stripes corresponds approximately to the size of the optic fibre arborizations on the Xenopus optic tectum 19,20

We thank R. V. Stirling for developing the method of HRP staining and Liz Hirst for histological assistance.

Received 3 December 1981, accepted 12 February 1982

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Adhesion-dependent heparin production by platelets

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It is well known that platelets are involved in blood clotting. In addition, substances of the platelet granules or on the platelet surface cooperate with plasma and vessel wall components to modulate control of haemostasis1. Here we provide evidence that platelets adhered to a substrate can release heparin.

When fresh human platelets were isolated from plasma and incubated in vitro in suspension or allowed to adhere to a solid surface, the medium of adherent cells showed an anticoagulant activity that was absent in the medium of suspended cells. As shown in Table 1, the production of the activity is adhesion dependent but does not require a specific adhesion substrate; however, artificial rafts containing compounds found in the subendothelial basal lamina were more effective than plastic or glass surfaces in inducing the release of the anticoagulant activity. This activity is resistant to heat, proteolysis, hyaluronidase and chondroitinase, is sensitive to nitrous acid, retained by anionic exchangers and eluted by cationic exchangers.

Figure 1a shows the electrophoretic analysis of platelet glycosaminoglycans. Chondroitin 4-sulphate is the only glycosaminoglycan present in suspended platelets, with sialosaccharides and trace amounts of dermatan sulphate. In contrast, in adhesion conditions, a complex pattern of glycosaminoglycans is present, the bands being identified as chondroitin 4-sulphate, sialosaccharides, dermatan and heparan sulphates

Table 1 Anticoagulant activity of media from platelets incubated in different conditions and sensitivity to various degradative agents

Culture conditions	Thrombin time (s)
No platelet	20/11
Suspended platelets	
Platelets adherent to glass, plastic or	
polyvinylchloride	120/150
Platelets adherent to artificial rafts containing	
type IV collagen, fibronectin and laminin	160/200

Type of degradation or treatment on glass-adherent platelet medium

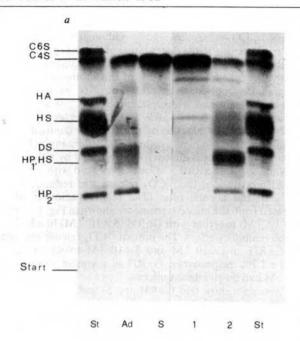
Exhaustive proteolysis, chondroitinase ABC, testicular hyaluronidase, heat (10 min at 100 °C) 120/160 passage through AG 50 X8 Nitrous acid, passage through AG 1 X2 30/40

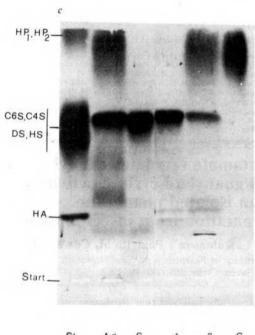
Platelets were isolated from pooled human blood containing either EDTA (0.1%) or sodium cytrate (0.38%) as anticoagulant. The blood was allowed to sediment spontaneously at room temperature and the upper phase was centrifuged at low speed (10g for 10 min). The supernatant was centrifuged at 1,000g for 2 min to sediment residual leukocytes and the platelets were then sedimented from the second supernatant by prolonged low-speed centrifugation (30 min at 160g). Leukocyte contamination was less than 1% as checked with the light microscope after May-Grunwald-Giemsa staining. Platelets were suspended in Hank's saline containing 1 mg ml⁻¹ glucose; the concentration was 3×10^8 cells per ml for the suspension experiments and 10-fold diluted for adhesion experiments. Cells were layered on a solid surface with a ratio of 3×10^6 cells per cm² and allowed to adhere to the substrate for 15 min. At the end of this incubation up to 60% of the cells were adherent. The medium was collected and centrifuged to remove non-adherent platelets. Artificial collagen rafts containing basement membrane non-collagenous proteins were prepared according to Rubin et al.3. All the matrix compounds were a gift of Dr R. Timpl. The media were concentrated 40-fold by ultrafiltration on Amicon apparatus equipped with a YM5 membrane and used directly for thrombin time assay. The test was carried out as follows: 100 µl of plasma pool were incubated at 37 °C with an equal volume of the tested sample, 100 µl of a thrombin solution (Boehringer-Mannheim) were added and the coagulation time was measured. Alternatively, media were digested with papain (0.1 mg ml⁻¹) at 60 °C for 48 h in the presence of 5 mM β -mercaptoethanol at neutral pH. After heating for 1 h at 95 °C to inactivate the protease, various aliquots were treated with hyaluronidase, chondroitinase or nitrous acid as described elsewhere After dialysis and concentration to the original volume, the processed samples were again assayed for anticoagulant activity by the same test. Aliquots were passed through cationic or anionic exchangers (Bio-Rad), neutralized, dialysed, concentrated to the original volume and tested for the anticoagulant activity.

Table 2 Pulse labelling of platelet glycosaminoglycans

Compound	C4S	HS/DS	HP
Densitometric value of the electrophoretic band (A units) H-glucosamine (c.p.m.) S-inorganic sulphate (c.p.m.)	0.37	0.15	0.32
	9	134	313
	7	49	127

Adherent platelet medium was added with $1\,\mu\text{Ci}\,\text{ml}^{-1}$ of $^3\text{H-glucosamine}$ and $5\,\mu\text{Ci}\,\text{ml}^{-1}\,^{35}\text{S-inorganic sulphate (NEN)}$ and the cells were incubated for 30 min Glycosaminoglycans were isolated as described in Fig. 1 legend, subjected to electrophoresis at pH 5.0 and densitometrically recorded. The cellulose acetate was cut into strips corresponding to the well resolved bands of chondroitin 4-sulphate (C4S) and heparin (HP) and the less resolved bands of heparan and dermatan sulphates (HS/DS). The acetate strips were then counted in a Bray's scintillation mixture.





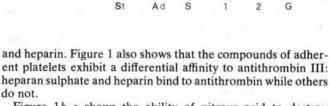
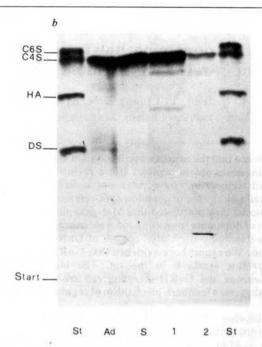


Figure 1b, c shows the ability of nitrous acid to destroy heparan sulphate and heparin as well as the high sulphate content of heparin. Commercial and platelet heparin was diluted to obtain solutions producing the same slowing effect on the thrombin time assay and the same volume of these solutions was then run on electrophoresis, stained and recorded by densitometry. A comparison of the peak areas showed anticoagulant activity of platelet heparin to be higher than that of the commercial standards.

When adherent cells were pulse labelled with radioactive precursors (see Table 2), chrondroitin 4-sulphate took up almost no label whereas the other compounds, particularly heparin, were labelled by both radioactive glucosamine and sulphate, suggesting synthesis or a post-synthetic processing of N-sulphated glycosaminoglycans.



a, Electrophoresis of glycosaminoglycans extracted from the media of adherent and suspended platelets. Electrophoresis was on cellulose acetate at pH 5.0 according to Cappelletti et al.⁵. b, Same samples as in a after direct nitrous acid treatment as described by Cappelletti et al.6, to identify N-sulphated compounds. c, Same samples as in a, run at pH 1.0 according to Wessler7, to reveal the relative sulphate content. After exhaustive proteolysis with papain the media were heated for 1 h at 95 °C and centrifuged at alkaline pH to avoid the formation of complexes between glycosaminoglycans and precipitated proteins. This is a critical step to avoid a selective loss of N-sulphated compounds. Supernatants were neutralized, loaded on a column of AG 50 X8 (Bio-Rad) and eluted with distilled water to remove the non-acidic proteins. Residual acidic proteins were precipitated from the eluate with 10% trichloroacetic acid. The glycosaminoglycans were recovered from the supernatant by precipitation with 2.5 volumes of cold ethanol. Cellulose acetate electrophoresis was carried out as described elsewhere5. Glycosaminoglycans from medium of adherent platelets were loaded onto a gel of antithrombin III-substituted Sepharose 4B (Pharmacia) following the instructions of the manufacturer: 10 mg of antithrombin III (a gift of Professor B. Casu) were coupled to 10 ml of agarose. The column was washed with 50 ml (7 column volumes) of 0.1 M Tris-HCl pH 7.4; retained glycosaminoglycans were eluted with 30 ml of 1.5 M NaCl in 0.1 M Tris-HCl pH 7.4. Both elution volumes were dialysed against distilled water and freeze-dried for the analysis of glycosaminoglycans. 1, Compounds not retained by antithrombin; 2, compounds retained by antithrombin. Letters to the left refer to the bands of the standard. The bands of the biological samples were characterized as described elsewhere4. Compounds not migrating at pH 1.0 were identified as sialosaccharides on the basis of the susceptibility to neuraminidase degradation. C6S, chondroitin 6-sulphate; C4S, chrondroitin 4-sulphate; HA, hyaluronic acid; HS, heparan sulphate; DS, dermatan sulphate; HP₁, HP₂, fast-moving heparin and slow-moving heparin as described by Bianchini et al.*; St, standard mixture; G, standard heparin (Glaxo).

Platelets are known to release several clotting factors on aggregation, including fibrinogen, thrombin, coagulation factor V and the anti-heparin platelet factor IV2. The present finding provides evidence of heparin release by a major blood cell subclass, thus indicating a possible source of endogenous heparin. Furthermore, it may indicate an alternative, nonthrombogenic role for platelets in vessel wall repair and control of clotting.

Received 29 September 1981; accepted 5 February 1982

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Inhibition of dopamine biosynthesis by gonadotropin-releasing hormone in rat

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There is evidence that the neuroendocrine system can be modulated by endogenous opioid peptides (for a review see ref. 1). Recently Rotsztejn et al.2 have ruled out a direct effect of Met-enkephalin on release of gonadotropin-releasing hormone (GnRH). Instead they postulated that Met-enkephalin inhibits the secretion of dopamine from dopaminergic neurones thereby reducing the dopamine-stimulated release of GnRH from the hypothalamus. We report here evidence that GnRH can itself supress dopamine synthesis in the rat. The fact that the dopamine neurone and GnRH-secreting cell are adjacent to each other³ suggests a feedback mechanism of regulating GnRH release.

Sprague-Dawley rats (180-230 g) were killed by decapitation. The corpus striatum was dissected on ice and homogenized in 10 vols 0.32 M sucrose using a Teflon pestle tissue homogenizer. After centrifugation at 1,000g for 15 min, 50 µl aliquots of the synaptosome-containing supernatant were incubated with 150 µl of physiological medium containing (mM): 125 NaCl; 1.48 CaCl₂; 4.8 KCl; 2.5 MgSO₄; 22 NaH₂PO₄; 10 NaHCO₃; 16 glucose, to give a final pH of 6.6 after equilibration with 95% O_2 -5% CO_2 at 37 °C. $[1^{-14}C]$ tyrosine (specific activity 50 mCi mmol⁻¹) at a concentration of 10 μ M was added to the preparation, together with bacitracin (10⁻⁵ M) to reduce the degradation of polypeptides. GnRH, Met-enkephalin or naloxone were added to the incubation medium with 10 μl 0.1 M phosphorous buffer pH 6.6 as carrier; controls recieved the buffer alone. The method used to determine dopamine synthesis was modified from that of Weiner⁴ and Kuzcenski⁵. who measured the release of ¹⁴CO₂ from [1-¹⁴C]tyrosine. We simplified the incubation medium to mimic the cerebrospinal fluid and used a respirometer to supply oxygen and allow continuous measurement of the ¹⁴CO₂ output from the tissue⁶. Addition of iodotyrosine $(5 \times 10^{-4} \text{ M})$ to the incubation medium inhibits >90% of the ¹⁴CO₂ release⁷.

Dopamine formation was calculated when the ¹⁴CO₂ output had reached a steady state, that is, after 50 min incubation. The rate of dopamine synthesis in 10 preparations was 0.2± $0.02 \text{ mol mg}^{-1} \text{ min}^{-1} \text{ (mean} \pm \text{s.d.)}.$

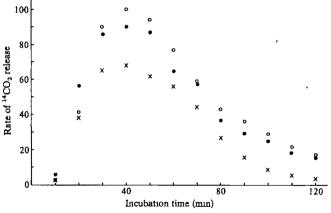


Fig. 1 Rate of ¹⁴CO₂ release from labelled tyrosine plotted against incubation time. The exponential increase in rate before 50 min of incubation is from isotopic equilibrium⁶. The rate of $^{14}\text{CO}_2$ release maintained steady-state values between 50 and 120 min of incubation. GnRH $(5 \times 10^{-6} \text{ M})$ added to the preparation (x) gave ~30% inhibition of dopamine synthesis. Naloxone at 5×10^{-6} M together with GnRH blocked 70% of this inhibition (•). O, Control.

Met-enkephalin (10⁻⁵ M) added to the incubation mixture reduced ¹⁴CO₂ release by ~40%. Although some of this inhibition could be due to the degradation of the enkephalin releasing unlabelled tyrosine, we showed by kinetic studies using added unlabelled tyrosine that >10% of this inhibition was a direct effect. Other opioid δ-receptor drugs, for example, D-Ala²-Met-enkephalin and Metkephamid⁸ at 10⁻⁵ M also inhibited dopamine synthesis by ~10% and 20% respectively and this inhibition was not blocked by naloxone. In contrast, the 11% inhibition of dopamine synthesis by sufentany¹ (10⁻⁵ M), a μ -receptor drug, was almost totally blocked by naloxone.

When GnRH (5×10⁻⁶ M) was incubated with the synaptosomal preparation, the $^{14}CO_2$ release was reduced to 73.4± 6.6% of the normal rate. (The relative amounts of $^{14}CO_2$ released from the added tyrosine are shown in Fig. 1.) Naloxone $(5\times10^{-6} \text{ M})$ together with GnRH $(5\times10^{-6} \text{ M})$ blocked ~70% of the inhibitory effect. The normal $^{14}CO_2$ release was reduced by GnRH at 1×10^{-6} M and 5×10^{-7} M to $83.1\pm3.8\%$ and $89.9\pm2.7\%$ respectively. GnRH at a concentration of $<1\times$ 10⁻⁸ M had no significant effect.

These data show that GnRH and δ - and μ -receptor opioids all have similar inhibitory effects on dopamine synthesis. We therefore postulate that GnRH exerts a negative feedback action on dopaminergic neurones, that is, GnRH inhibits it own release by inhibiting dopamine synthesis.

This work was partially supported by National Science Foundation R.O.C.

Received 14 October 1981, accepted 11 February 1982

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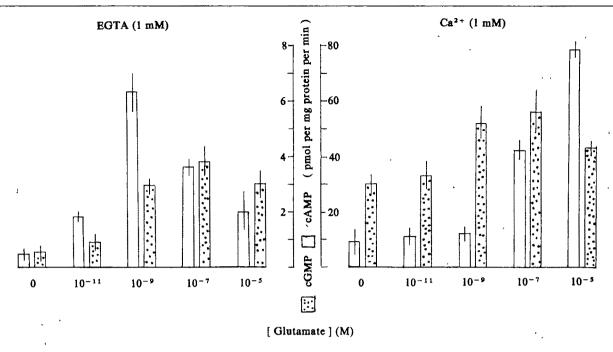
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Glutamate regulates adenylate cyclase and guanylate cyclase activities in an isolated membrane preparation from insect muscle

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It is generally believed that glutamate serves as a neurotransmitter at many vertebrate and invertebrate synapses. There is, however, little information concerning the possible involvement of cyclic nucleotides as intracellular second messengers for any of the postsynaptic actions of glutamate. Direct activation of adenylate cyclase has been reported for several neurotransmitters and neuromodulators¹, but no such effect has been reported for glutamate. Moreover, although several neurotransmitters including glutamate have been shown to increase cyclic GMP levels in intact preparations of nerve and muscle tissue^{2,3} apparently by promoting Ca²⁺ entry into the cells, no neurotransmitter has been shown to activate guanylate cyclase in a cell-free preparation. L-Glutamate is the prime candidate for the neurotransmitter at the excitatory neuromuscular. synapses in insects4. Moreover, recent studies have shown that glutamate application raises both cyclic AMP⁵ and cyclic GMP levels (P.M.C., unpublished observations) in insect muscle. To determine the mechanism underlying these increases, we have studied the effect of glutamate in a membrane fraction prepared from this muscle, and report here that the neurotransmitter activates both adenylate cyclase and guanylate cyclase in the membrane preparation, and that the activation by glutamate of guanylate cyclase is calcium independent.

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Fig. 1 Glutamate stimulation of cyclic AMP (cAMP) and cyclic GMP (cGMP) production by a membrane preparation from the body-wall muscles of the dipteran Sarcophaga Third instar larvae of the insect were used. The central nervous system, digestive system and fat body were removed and the body-wall muscles were scraped from the cuticle and then homogenized on ice in isotonic saline (30 larvae per 25 ml) (NaCl, 120 mM; KCl, 2 mM; CaCl₂, 0.5 mM, thiourea, 1 mM; Tris-HCl, 10 mM, pH 6 8) in a Teflon/glass homogenizer (10 passes at 100 r.p.m.). The homogenate was centrifuged at 4,000g for 30 min and the supernatant was collected and centrifuged at 280,000g for 60 min. The supernatant (S2) was removed and the pellet (P2) lysed by resuspension in 25 ml of hypotonic saline (10 mM Tris, 1 mM thiourea, and either 0.5 mM CaCl₂ for 'calcium' incubations or 1 mM EGTA for calcium-free incubations). The suspension was then repelleted by centrifugation at 280,000g for 60 min. The pellet formed (P3) was resuspended in 2 ml cyclase assay buffer (10 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 0 5 mM MnCl₂, 0.05 mM alanine, 1 mM thiourea and either 0.5 mM CaCl₂ or 1 mM EGTA, pH 6 8), adenylate and guanylate cyclase activities were assayed concurrently and the cyclase reaction started by the addition of an equal volume of cyclase assay buffer containing 2 mM ATP, 1 mM GTP and 2% albumin Incubations were stopped by the addition of five vol of acidified alcohol (0.5 ml M HCl in 100 ml ethanol). Samples were evaporated to dryness at 55 °C under nitrogen and then extracted in extraction buffer (10 mM Tris, 1 mM EDTA pH 6 8). Cyclic AMP was assayed by the method of Brown et al. Ocyclic GMP was assayed by radiommunoassay as described elsewhere. The protein content of samples was determined using the Lowry folin-phenol method. Each point represents the mean ±s.e.m. of at least three estimations. In control experiments it was found that calcium was required during the initial homogenization to retain cyclase activity, whereas subsequent l

Figure 1 shows the effect of various concentrations of glutamate on production of cyclic AMP and cyclic GMP by a membrane preparation of body-wall muscle from the dipteran Sarcophaga. Adenylate cyclase activity was stimulated in either the presence or absence of calcium. However, stimulation of cyclic AMP synthesis required a lower concentration of glutamate in the absence of calcium (activation occurring at a glutamate concentration >10⁻¹¹ M) than in the presence of calcium (>10⁻⁹ M glutamate). In the absence of calcium, maximal stimulation of adenylate cyclase activity was observed at ~10⁻⁹ M glutamate, inhibition occurring at higher concentrations of the neurotransmitter. Guanylate cyclase activity was also stimulated in either the presence or absence of calcium, maximal stimulation being observed at 0.1 µM glutamate in both cases. The similarity of the dose-response curves as a function of glutamate concentration in the absence and presence of calcium (Fig. 2) indicate that this enzyme activity can be stimulated by either of two mechanisms, one of which is glutamate dependent and calcium independent, the other being calcium dependent and glutamate independent.

The fact that very low concentrations of glutamate are effective in stimulating adenylate cyclase and guanylate cyclase activities suggests that these intracellular messenger systems could be activated by the very low concentrations of transmitter released during spontaneous quantal discharge⁶.

Enzyme activity was proportional to incubation time for both guanylate cyclase (Fig. 3) and adenylate cyclase (data not shown) for glutamate concentrations between 10⁻¹¹ M and 10⁻⁵ M, both in the presence and absence of added calcium.

As the present results represent the first evidence of the activation of guanylate cyclase by any neurotransmitter in a

broken cell preparation, the production of cyclic GMP from GTP following glutamate treatment was verified using an alternative assay procedure in which $[\alpha^{-32}P]$ GTP was used as the substrate. Essentially similar results were obtained (Fig. 4).

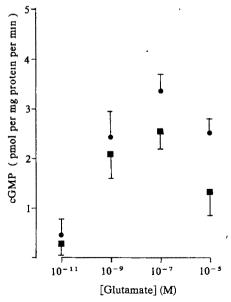


Fig. 2 Increase in guanylate cyclase activity after L-glutamate stimulation in the presence and absence of calcium Membrane preparations were isolated, incubated and cyclic GMP extracted as described in Fig. 1 legend

•, EGTA (1 mM), •, ca²⁺ (1 mM).

HPLC followed by TLC of control and phosphodiesterasetreated cyclic GMP' column eluate fractions, confirmed that the radioactive product was 3', 5'-cyclic GMP. Glutamate stimulation of adenylate and guanylate cyclase activities has also been observed for an isolated membrane preparation prepared from the body-wall muscles of a second species of dipteran insect, Lucilia sericata (data not shown).

We have made a preliminary investigation of the pharmacological specificity of adenylate and guanylate cyclase. The neutral amino acid alanine was found to be totally inactive in stimulating either adenylate or guanylate cyclase activities. However, inclusion of alanine in the incubation medium reduced considerably the level of nonspecific interference in the cyclic GMP radioimmunoassay, therefore alanine was included at a low concentration in the assay buffer, α -Ketoglutaric acid, a precursor of L-glutamate, was also found to have no stimulatory effect. The structurally related amino acids aspartate and cysteine sulphinate were also tested and found to possess agonist activity but of a lower potency than glutamate. We also determined the cation requirements necessary for measurable cyclase activity. Although Ca2+ is not required in the final incubation medium, it must be included in the isolation buffer. Preliminary studies suggest that either Mg²⁺ or Mn²⁺ may serve as the major cation in the cyclase assay buffer.

Although various authors^{7,8} have reported stimulation of guanylate cyclase activity by calcium in broken cell preparations, and Kakiuchi and co-workers9 have shown that in Tetrahymena pyriformis calcium activation of membrane-bound guanylate cyclase is calmodulin dependent, the present report of glutamate activation of guanylate cyclase in a broken cell preparation is in sharp contrast to the failure in both our own and various other laboratories to demonstrate activation by neurotransmitters of guanylate cyclase in cell-free preparations. Our present results agree with previous observations in that guanylate cyclase can be activated by calcium, but differ from previous reports in that we have demonstrated an additional glutamatesensitive component which may be activated by this neurotransmitter in the absence of calcium. It is not clear whether the neurotransmitter-sensitive guanylate cyclase reported here is unique to insect muscle or whether it represents a more general

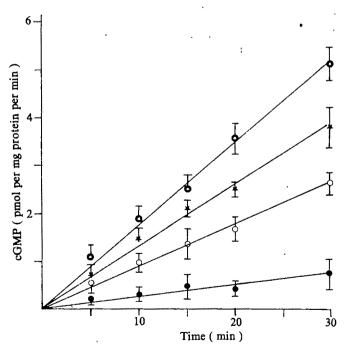


Fig. 3 Time of production of cyclic GMP by an isolated muscle membrane preparation in the presence and absence of added glutamate. Membrane preparations were isolated, incubated and cyclic GMP extracted as described in Fig. 1 legend, except for the variation in incubation time. ●, EGTA (1 mM); ○, EGTA (1 mM)+glutamate (10⁻⁷ M); ★, Ca²⁺ (1 mM), ☆, Ca²⁺ (1 mM)+glutamate (10⁻⁷ M).

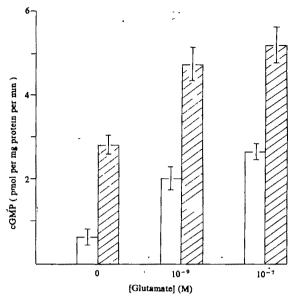


Fig. 4 Production of $[\alpha^{-32}P]$ cyclic GMP from $[\alpha^{-32}P]$ GTP. Membrane preparations were isolated and incubated as described in Fig. 1 legend except that $[\alpha^{-32}P]$ GTP (0.05 mol per mCl) was used as the substrate for the guanylate cyclese and the cyclic GMP produced during incubation was accordingly labelled with ³²P Incubations were terminated as described in Fig. 1 legend and after evaporation to dryness, samples were extracted with 1 ml extraction buffer containing 1 nmol cyclic GMP to act as a marker during HPLC. A 0.5-ml sample was introduced to the chromatography column (Whatman SAX; 10×30 cm) and the chromatograph developed by isocratic elution using 0 005 M NaH₂PO₄ buffer, pH 4 2. The column cluate was continuously minitored for UV absorbance at 254 nm and 500-µl samples collected using a Gilson fraction collector and monitored for radioactivity. The 'cyclic GMP' peak as determined by the UV absorbance of the added standard was found to correspond to a peak of radioactivity and the yield of $[\alpha^{-32}P]$ cyclic GMP calculated by determining radioactivity recovered from the column after injection of radioactive standards. Representative samples of 'cGMP' column fractions were pooled and evaporated to dryness under N₂ at 55 °C. Samples were then taken up in a small volume (20 μ l) of distilled water; 5 μ l was applied directly to TLC plates (silica gel GF, 250 μ m, with fluorescent marker) and the remainder incubated for 30 min with a high concentration of 3'5'-cyclic nucleotide phosphodiesterase (Sigma) This incubation was stopped by adding 5 vol of acidified ethanol. Samples were again evaporated to dryness under nitrogen at 55 °C and then taken up in 15 µl distilled water; a 5-µl sample was applied to a TLC plate together with the untreated sample and suitable cyclic GMP and GMP standards. The chromatogram was developed in n-butanol/ethyl/acetate/methanol ammonia/water (5:3.6.4:2). The developed chromatogram was monitored for UV absorbing spots and then 1-cm strips were scraped off and the radioactivity counted in a liquid scintillation counter This procedure confirmed that the radioactive product co-chromatographed with cyclic GMP after phosphodiesterase treatment co-chromatographed with GMP. Open columns, with added EGTA, cross-hatched columns, with added Ca2+.

system present in other tissues for which appropriate assay conditions have not yet been found.

This work was supported by USPHS grant MH-17387, a grant from the McKnight Foundation (ns 68440) and a British Science Research Council Fellowship to N.L.R.

Received 8 June 1981, accepted 7 January 1982

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Single Ca²⁺-activated nonselective cation channels in neuroblastoma Gary Yellen

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Recent work suggests an important role for intracellular agents in controlling ion channels in the membranes of nerve cells and other excitable tissues. Calcium ions^{1,2}, cyclic nucleotides³ and protein kinases4,5 can all act on the inner surface of the membrane to influence ion channel activity. Earlier studies of these effects on intact cells could not, however, effectively control or measure conditions on the inner membrane surface. The technique of recording from detached membrane patches^{6,7} permits free access to the intracellular face of ion channels and makes it possible to study them in isolation from the many components of the cytoplasm. With this technique, I have studied the response of membrane patches from neuroblastoma cells to intracellular Ca2+ ions, and have found a class of nonselective cation channels activated by micromolar concentrations of Ca2+ on the intracellular face of the membrane. These channels are almost equally permeable to Na⁺, K⁺, Li⁺ and Cs⁺ ions, but are practically impermeable to Ca²⁺ ions. Similar channels were first found recently in cultured heart cells, where they probably account for the previously reported 'transient inward' current⁵. Their discovery in neuronal cells as well as heart cells suggests that this hitherto scarcely recognized channel species may be more widely distributed than previously supposed.

Voltage-clamp recordings were from inside-out^{6,7} and outside-out membrane patches of mouse neuroblastoma cells (N1E-115). Inside-out patches have their intracellular face exposed to the bath, while outside-out patches have their extracellular face exposed to the bath. The exposed surface can be readily superfused. Cells were subcultured onto glass coverslips 2-7 days before use; some cells were treated with prostaglandin E₁ (PGE₁) (10 µM; P-L Biochemicals) 2 days after subculture (L.-Y.M. Huang, personal communication). Voltage-activated Na⁺ channels were observed in most patches. Normal behaviour of Na+ channels served to indicate that patches were not sealed over; rounded channel currents usually indicate that the membrane has sealed over to form a closed vesicle7. Other channels observed were Ca2+-activated K+selective channels and various voltage-sensitive outward current channels. The Ca²⁺-activated nonselective channels were observed in about 1 out of 20 patches from cells not treated with PGE₁, and about 1 out of 5 patches from treated cells.

Channel opening and closing events like those in Fig. 1 were seen in inside-out or outside-out patches when the intracellular face was bathed in 1-50 μ M Ca²⁺. These currents had a reversal potential close to 0 mV in a variety of ionic conditions (see below), and a conductance of 22 ± 1.5 pS (s.e.m., n=6) at 24 °C, with 125 mM NaCl outside and 125 mM KCl inside.

Channel opening events occur in clusters, and their kinetics indicate a complex scheme for channel gating. A cluster of channel openings and closings may last on the order of 30 s. Within such a cluster, there are opening events lasting on the order of 50-200 ms. One of these opening events may be interrupted by brief closing events (see, for example, the -70 mV trace in Fig. 1). Clusters of openings are sometimes separated by prolonged closings of many minutes. This

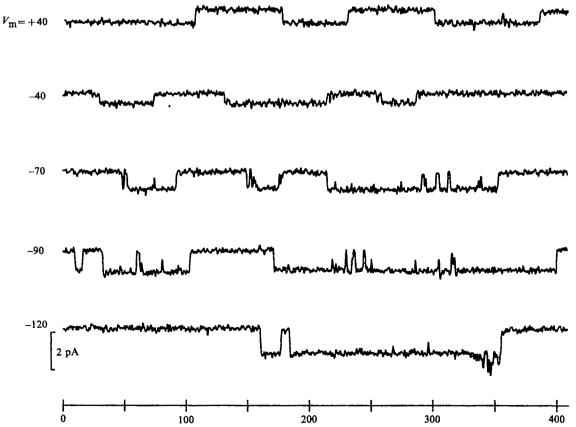


Fig. 1 Ca²⁺-activated nonselective cation channel currents. Outward current is always defined as current from the intracellular to the extracellular side of the membrane, and is indicated as upwards. Membrane potentials are always specified as the potential on the intracellular side of the membrane, relative to the extracellular side. Channel currents are outward in the +40 mV trace, and inward in all the traces at negative membrane potentials. Records are from an outside-out patch. Extracellular (bath; in mM). 125 LiCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, 70 dextrose, pH 7.2. Intracellular (pipette). 125 NaCl, 1 MgCl₂, 10 HEPES, 70 dextrose, 5 EGTA+4.925 CaCl₂ (free [Ca²⁺]=10 μM), pH 7.2. All Ca²⁺-EGTA buffers were computed with a K_{app} (at pH 7.2) = 1.48×10⁻⁷ M (ref. 13, corrected for hydrogen ion activity). Temperature 22 °C. Low-pass filtered at 500 Hz with an 8-pole Bessel characteristic.

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behaviour indicates gating which is more complex than a single first-order transition between an open and a closed state. Gating of the channels is not strongly voltage dependent over a range from -120 to +80 mV.

Intracellular Ca^{2+} concentrations of $\geqslant 1 \mu M$ reversibly activate these channels (Fig. 2a), as can be seen by changing $[Ca^{2+}]$ on the exposed (intracellular) surface of an inside-out patch. If Ca^{2+} is removed (solution changed to 5 mM EGTA, 0 Ca^{2+} , 1 mM Mg^{2+}), the channel openings become less frequent and eventually cease (complete disappearance sometimes takes 1–2 min). When intracellular Ca^{2+} is restored, the channels begin to open almost immediately. This whole process can be repeated several times on a single patch, using the perfusion system shown in Fig. 2b. Solutions can be changed rapidly by moving the patch pipette among several continuously flowing perfusion streams.

The channel permeation selectivity was studied in outside-out patches, with 10 or 50 µM free [Ca2+] on the intracellular side (that is, in the pipette) to activate the channels. Two results argue against a significant anion permeability of the channels. First, replacing all but 3 mM of Cl by aspartate on one or both sides of the membrane changed neither the reversal potential nor the unit conductance. Aspartate is a much larger anion than chloride, and might be expected not to pass through the channels as easily. Second, channel currents in the presence of a salt gradient show approximately perfect selectivity for cations. In a fivefold salt gradient (25 mM NaCl extracellular, 125 mM NaCl intracellular; osmolarity preserved with dextrose), a perfectly cation-selective channel should reverse at -41 mV, and a perfectly anion-selective channel at +41 mV (22 °C). In such conditions, I found that these currents reverse within 2 mV of -41 mV.

Several alkali metal cations and Ca^{2+} were tested for permeation through the channel. All of the alkali metals permeated nearly as well as Na^+ . To obtain a complete current-voltage (I-V) relationship over a wide range of membrane potentials, a voltage ramp was applied to the membrane. Figure 3a shows a single record of membrane current in response to such a ramp (average leak current has been subtracted). When the channel opens, the current changes abruptly from the leakage level (zero in the leak-subtracted records) to the open channel level. For as long as the channel remains open, the current follows the I-V relationship for the open channel. A complete I-V relationship for the open channel may be obtained by extracting all the open channel segments from many records and averaging

them point by point for each voltage (points from one voltage are averaged only with other points from the same voltage, see Fig. 3b). The leakage current is obtained by a similar process.

The ramp clamp method has several advantages over measuring channel currents at many fixed potentials. Channel openings are small and difficult to see at fixed potentials near their reversal potential, but can readily be seen in ramp clamp records as an inflection in the slope of the current. Also, although it is sometimes difficult to distinguish between the opening of an inward current channel and the closing of an outward current channel at a fixed potential, the open channel is unambiguously identified in a ramp clamp by the increased slope of the I-V curve. Finally, a complete, continuous I-V curve can be obtained quickly, and without holding the membrane at depolarized potentials for prolonged periods (which often causes membrane breakdown). Open channel I-V curves derived from ramp clamp data agree well with those derived from measurements of fixed-potential records. Ascending and descending ramps give similar results.

The open channel I-V curve in symmetrical 125 mM Na⁺ has a slope conductance at 0 mV of ~ 17 pS (22 °C), and shows a small outward rectification at positive potentials (Fig. 3b). K⁺ goes through the channel just as easily as Na⁺: the reversal potential with Na⁺ outside and K⁺ inside is still 0 mV. Cs⁺ and Li⁺ show only slightly lower permeability than Na⁺ and K⁺, with a reversal potential of -8 mV (Cs⁺ or Li⁺ outside, Na⁺ inside) and a slope conductance at hyperpolarized voltages of ~ 12 pS. Ca²⁺ (82 mM, outside; Na⁺ inside) has a very low permeability (if any); the channel current remains outward over a range of hyperpolarized voltages (Fig. 3b).

Neuroblastoma cells have at least one other type of Ca²⁺-activated channel, a Ca²⁺-activated K⁺ channel (unpublished observations). This channel is similar in its properties to the Ca²⁺-activated K⁺ channels described by Marty¹⁰ in chromaffin cells and by Pallotta, Magleby and Barrett¹¹ in cultured rat muscle cells. Even though the Ca²⁺-activated K⁺ channels have about 10 times the conductance of the Ca²⁺-activated nonselective channels, they are much more selective in their permeation.

Recently, it was reported that pressure-injection of CaCl₂ in snail neurones evokes an inward current¹². However, this Ca²⁺-activated inward current is not affected by replacing all the extracellular NaCl with sucrose, and may thus have a different ionic basis from the Ca²⁺-activated nonselective cation channel currents described here.

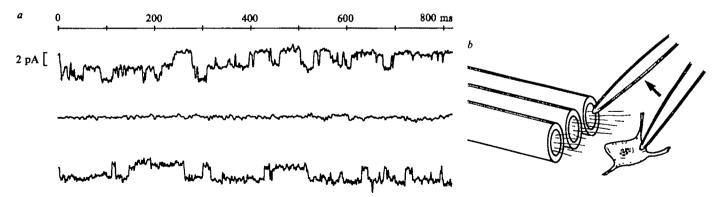
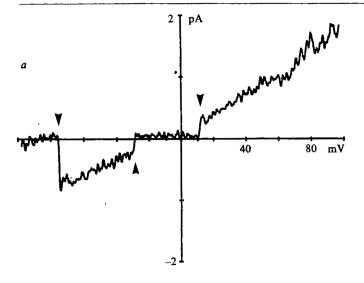


Fig. 2 a, Ca²⁺-dependent activation of channel currents. A series of records was collected with different intracellular [Ca²⁺]. Top trace: channel currents with 1 μM free [Ca²⁺] solution perfusing the intracellular side. Middle trace: current record after 2 min perfusion with 0 free [Ca²⁺] solution (that is, 5 mM EGTA, no added Ca²⁺). Bottom trace: channel currents immediately after return to 1 μM free [Ca²⁺]. Solution changes were performed using a perfusion system like that shown in b. Temperature 25 °C; 300 Hz filtering. Data are from an inside-out patch with Na⁺ saline outside (divalent ions: 3 mM MgCl₂, 0.1 μM free [Ca²⁺] (5 mM EGTA buffer)) and K⁺ saline inside (divalent ions: 1 mM MgCl₂, 1 μM free [Ca²⁺] or 0 free [Ca²⁺] (5 mM EGTA buffer)). Membrane potential is -60 mV. b, Diagram of perfusion apparatus for rapid solution changes. Solutions flow continuously out of perfusion pipettes (i.d. -70 μm) into the bath at a rate of 5-50 linear μm s⁻¹ (estimated from the velocity of small particles in the centre of the flow stream). After detaching the membrane patch from the cell, the patch pipette is moved completely into the outflow of one of the perfusion pipettes. Solution changes can be readily performed by moving the patch pipette into a different perfusion stream. Moving the pipette takes about 1 s; the solution change at the membrane is quite rapid, as judged from the immediate response of Ca²⁺-activated K⁺ channels to perfusion of Ca²⁺ solutions. This apparatus was used for changing internal [Ca²⁺] in experiments with inside-out patches.



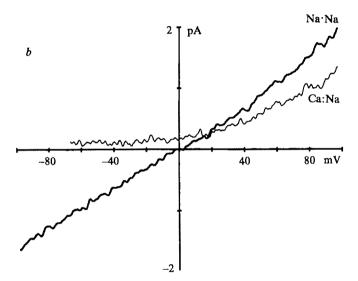


Fig. 3 Open channel I-V relationship obtained with ramp voltage clamp. a, Single current record from outside-out patch with Li⁺ saline outside, Na⁺ saline (with 10 μM free [Ca² The average leak current from 32 records has been subtracted (averaging method is described in the text). The arrows indicate channel transitions: the first arrow indicates a channel opening, the second a closing, and the third an opening. The residual current when the channel is closed is due to imperfect leak subtraction. A ramp from -100 to +100 mV was performed in 410 ms. Temperature 22 °C; 300 Hz filtering. b, Average open channel I-V curves. Open channel segments of records like that in a were identified to the computer, which averaged these segments point by point for each voltage. An average leakage I-V was subtracted from the open channel I-V (the leakage I-V was also collected by selective averaging, and, in this experiment, the leakage current was smaller than a single open channel current). The Ca:Na I-V was obtained with (in mM): 82 CaCl₂, 10 HEPES, 70 dextrose, pH 7.2 extracellular, and Na⁺ saline (10 μ M free [Ca²⁺]) intracellular. Temperature 22 °C. In measurements of the Ca:Na I-V, channel opening events were observed at potentials as low as -10 mV (size was +0.2 pA); below this potential, the currents were obtained from the average of several records which appeared to have a channel open during the whole trace.

Channels very similar to those described here have recently been found in primary-cultured rat heart cells8. They are activated by intracellular Ca²⁺, not much affected by voltage, have a size of ~23 pS at 22 °C and pass Na⁺ and K⁺ equally well. They may be responsible for the 'transient inward' current which produces oscillatory depolarizations (causing arrhythmias) in heart cells exposed to high Ca²⁺ or to Na⁺-K⁺-pump inhibitors9. The finding that these channels, appreciated only

for their role in the physiological dysfunction of cardiac cells. also appear in neuronal cells suggests that this channel species may be more widespread in both its occurrence and significance.

I thank Dr D. P. Corey for advice and encouragement, and Dr C. F. Stevens for both moral and financial support. This work was supported by NIH grant NS 12961. G.Y. is a NSF Predoctoral Fellow.

Received 20 November 1981, accepted 28 January 1982

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Human tumour antigens defined by cytotoxicity and proliferative responses of cultured lymphoid cells

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The long-term goal of many laboratories has been to develop cellular reagents having specific reactivity against human tumour cells. Such immune cells should prove useful for defining the antigenicity of human malignancies and may have important therapeutic potential, as has been clearly shown in some animal models¹. Here we describe methods of initiating continued lymphocyte cultures (CLC) having specific anti-tumour reactivity using conditioned media containing interleukin-2 (IL-2).

Direct cytotoxicity assays show that ~30% of patients have specific reactivity against autologous tumours²⁻⁴. It was anticipated that such in vivo sensitized cells would express receptors for IL-2 and thereby be selectively susceptible to growth in vitro in the presence of IL-2 (ref. 5). Blood lymphocytes were prepared from heparinized blood samples taken from cancer patients on the morning of surgery, before the administration of any narcotic agent. Mononuclear cells were isolated by flotation on Ficoll-Hypaque (Lymphocyte Separation Medium, Litton Bionetics, Maryland). Adherent cells were removed by culture in flasks (75 cm²) at 37 °C overnight. The lymphocytes (3×10⁵ ml⁻¹ in RPMI+20% heat-inactivated pooled normal human serum) were grown (without in vitro stimulation with tumour cells) in optimal quantities of different conditioned media containing IL-2 reactivity. The resulting cultured cells were tested for cytotoxicity against a range of target cells, including freshly isolated autologous and allogeneic tumour cells. CLC were also tested against the K562 cell line to determine the possible presence of natural killer (NK) cells. The cultured cells showed cytotoxicity against both autologous and allogeneic tumour targets and, in at least some conditioned media, high levels of cytotoxicity were also found against K562 (Table 1). There was no evidence of selective reactivity against the autologous tumour alone and frequently, cytotoxicity against allogeneic tumours of unrelated types exceeded that against autologous tumours. Similar data have been obtained by other workers⁶⁻⁹. The results are compatible with the presence within such cultures of several different types of effector, including polyclonally activated T¹⁰⁻¹² and natural killer (NK) cells 10,13

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Table 1 Cytotoxicity of lymphocytes from cancer patients after culture for 21 days in conditioned media containing IL-2

Patient (site of tumour)	% Cytotoxicity against.			
	Type of medium	Autologous tumour	Allogeneic tumour	K562
Tu 3 (ovary)	H-CM	3.3	10.9*	51.6*
Tu 4 (parotid)	H-CM	28 9*	35.5*	87.6*
Tu 5 (parotid)	H-CM	8.6*	4.0	75.0*
Healthy donor	S-CM	15.9*	18.7*	31.3*
Tu 85 (lung)	S-CM	15.9*	18.7*	31.3*
Tu 58 (colon)	S-CM	13 6*	0	0
Tu 55 (colon)	S-CM	8.5*	33 7*	23.8*
Tu 131 (parotid)	LF-CM	18.4*	18.2*	0
Tu 57 (colon)	LF-CM	18.4*	5.2	8.1*
Tu 139 (gastric)	LF-CM	10 6*	20 8*	2.8

H-CM, from thoracic duct lymphocytes (phytohaemagglutinin (PHA) content .04 µg ml⁻¹ by haemagglutination); S-CM, from spleen lymphocytes (PHA 0.04 µg ml-1 content 2 µg ml 1), LF-CM, from tonsillar lymphocytes depleted of PHA by passage through an immunoabsorbent column of rabbit anti-PHA (no PHA detectable). Tumour tissue was received within 3 h of removal from patients Tumour cells, tumour-infiltrating lymphocytes and macrophages were isolated from enzymatically dispersed cell suspensions by methods described in detail elsewhere²¹. All populations showed high viability (>95% by Trypan blue exclusion) and contamination with 'inappropriate' cells was <10% as judged by morphological examination and histochemical staining1,20. Preparations were either used unmediately as stimulators in MLTC, or frozen in RPMI 1640 supplemented with 20% human serum and 10% dimethyl sulphoxide (2.5×106 cells in 2 ml) by constant-rate freezing at 1 °C min⁻¹ to -30 °C and stored over liquid nitrogen. Allogeneic tumours were, as far as possible, matched for cytotoxicity testing with the autologous tumours with regard to tumour site and histology. Cytotoxicity at effector/target ratio of 5 · 1 was determined after overnight incubation in fresh medium¹³, for each case.

*P<0.05, Mann-Whitney U-test.

In an attempt to induce more specific reactivity, blood lymphocytes were sensitized in vitro in mixed lymphocyte-tumour cultures (MLTC). Responder cells (1×10⁷ in 10 ml RPMI plus 20% human serum) were mixed with irradiated (3,000 R) tumour stimulators (2×106 in 2 ml medium) and cultured at 37 °C for 6 days in 25 cm² culture flasks kept upright. MLTC was considered positive when mean 3H-thymidine incorporation of stimulated cultures exceeded by at least threefold that of controls and clear dose-dependence of stimulation was apparent, and when blast cells were recognizable in bulk cultures. The factors critical for induction of high frequency of stimulation by autologous tumour cells have been considered elsewhere¹⁴. In the present experiments, positive MLTC was defined in 15 of 22 cases. Surviving lymphocytes were expanded by adding IL-2-containing media as described above, and these (MLTC-CLC) were tested for cytotoxicity after 10 and 21 days in culture. Typical results for such cultures are shown in Fig. While some preferential reactivity towards autologous

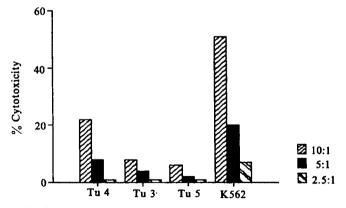


Fig. 1 Cytotoxicity of MLTC-positive cells grown in H-CM containing IL-2 for 17 days. Effectors were cultured in the absence of IL-2 for 24 h before testing and tested in a single assay at effector/target ratios of 10:1, 5:1 and 2.5:1 against the autologous tumour Tu 4 (parotid), and allogeneic tumours Tu 3 and 5 (ovarian ascites). The K562 cell line was used in measures of NK activity; cytotoxicity against the latter represented the major cytolytic potential in most of these cultures. Spontaneous ⁵¹Crrelease was: Tu 4, 21%; Tu 3, 11%; Tu 5, 14%; and K562, 7%.

tumours was induced in MLTC-positive cultures, CLC still had significant reactivity against allogeneic tumours and high cytotoxicity against K562. Thus, they were not appreciably more selective in their reactivity than unstimulated CLC.

To select for specific anti-tumour reactivity, blasts were isolated from MLTC on day 6 of culture on discontinuous gradients of Percoll in RPMI plus 20% human serum. Gradients were prepared in 15-ml tubes (No. 2095, Falcon Plastics, Maryland) and comprised 40% Percoll (5 ml); 35% (1.5 ml); 31.5% (2.5 ml) and 26% (2.5 ml). Samples were applied to the gradient in 1 ml medium and centrifuged at 550g for 30 min at room temperature. Blast cells were recovered from the 31.5% interface, lower numbers (contaminated with small lymphocytes) also being found at the 35% interface. Cells were washed twice in RPMI 1640 and cultures initiated at a concentration of 3×10^5 cells ml⁻¹ in RPMI plus 20% human serum supplemented with 10% conditioned medium containing IL-2 activity. These MLTC-blast-CLC were tested for cytotoxicity (4 h ⁵¹Cr-release assay) and proliferation after 8-29 days of culture in conditioned medium. Of 15 MLTC-blast-CLC, 13 showed selective lysis of autologous tumours (Table 2). Reactivity was apparent at low effector/target ratio and was dose dependent. By contrast, significant reactivity was only rarely recorded against at least two allogeneic tumour targets (Table 2). In many cases, cross-reactivity tests showed that the control targets were susceptible to lysis by appropriate autologous MLTC-blast-CLC. Following blast isolation, many cultures

Table 2 Cytotoxic reactivity of MLTC-blast-CLC grown in H-CM

		% Cytotoxicity (effector/target ratio, 5.1) against:		
Tumour no.	Diagnosis	Autologous tumour	Allogenesc tumour (no.)	K562
7	Lung cancer	28 4*	2.0(11)	10.1*
8	Cancer of gingiva	100*	5.7 (7)	19*
11	Lung cancer	23.7*	4.5 (7)	0
12	Melanoma	18 4*	1.4 (11)	7
15	Lung cancer	38.5*	1.4 (17)	1
17	Lung cancer	42.4*	0.4 (15)	2.7
25	Breast cancer	34.6*	8.7 (26)	21.8*
26	Breast cancer	15.5*	2.2 (25)	16.3*

Spontaneous release of 31 Cr from fresh tumour cells was <25% in a 4 h assay; K562 showed spontaneous release between 11% and 21% of total. Tests were performed on the same day against autologous tumour, blood monocytes and PHA-induced lymphoblasts and against at least two allogeneic tumour preparations matched, as far as possible, for tumour site and histology. No significant cytotoxicity was found against six autologous lymphoblasts (cytotoxicity -5.1 to 4.7%), but was evident against 1/7 blood monocytes (tumour 12, 11.4%) and in 4/41 cross-reactivity tests with 15 MLTC-blast-CLC against allogeneic tumour (-4.3% to 18.6% cytotoxicity). *P<0.05.

showed only low levels of cytotoxicity against the K562 cell line. The ability to reduce NK-like activity by density separation facilitated the interpretation of MLTC-blast-CLC killer function against autologous tumour. However, as an additional control, CLC were initiated from blasts induced by co-cultivation of tumour cells with allogeneic lymphocytes in seven cases. Five allogeneic MLTC-blast-CLC showed significant proliferation and cytotoxicity that was specific for the inducing tumours and levels of lysis were similar to those of autologous MLTC-blast-CLC against the same targets. Some cultures (7, 8, 25 and 26 of Table 2) continued to show significant NK activity against K562. The reason for the variability of NK levels among different CLC is not clear but cannot be related to levels of proliferation of particular MLTC.

Proliferative responses of MLTC-blast-CLC were assessed in primed lymphocyte tests. Cultures were restimulated with autologous and allogeneic cells in 48-h tests; 15 MLTC-blast-CLC showed a significant increase in ³H-thymidine uptake on restimulation with autologous tumour cells. The results of a series of assays are given in Table 3. MLTC-blast-CLC from a patient with squamous cell carcinoma (SCC) of the lung showed significant proliferation after restimulation with

Table 3 Primed lymphocyte test of MLTC-blast-CLC 17 (squamous cell

	No. of responders per well			
Stimulator	1×104	5×10 ³	2.5×10^{3}	1.25×10^3
CLC alone	1,115	530	620	360
Tumour 17	7,302*	9,314*	5,781*	4.069*
SCC 16	3,447*	2,100*	2,687*	561
Tumour macrophages 17	4,443*	1,553*	698	187
Monocytes 17	892	649	549	304
Lymphocytes 16	1.022	1,241	1.145	736
Lymphocytes 15	1,625	1,319	1,448	480

Restimulation of MLTC-blast-CLC 17 with autologous (tumour, monocytes and macrophages 17) and allogeneic cells. Decreasing numbers of responder cells were cultured for 48 h with a constant number $(5\times10^4 \text{ per well})$ of stimulator cells. Uptake of ³H-thymidine was measured after a 6-h pulse with 0.5 μ Ci radiolabel per well. Uptake of ³H-thymidine by stimulators was <100 counts per well and this value was subtracted from stimulated cultures. Stimulators tested were autologous tumours (squamous cell carcinoma of lung), autologous tumourderived macrophages, autologous blood monocytes, allogeneic squamous cell carcinoma of lung, and lymphocytes from allogenesc tumour donors 16 (ref. 15). Further testing of the MLTC-blast-CLC 17 line showed that it was stimulated neither by four allogeneic lymphocytes nor by eight allogeneic non-SCC tumours. * P < 0.05, Student's t-test.

autologous tumour. Less proliferation was apparent with tumour macrophages as stimulators and no increased 3Hthymidine incorporation was induced by autologous blood monocytes, lymphocytes or a panel of allogeneic lymphocytes and monocytes. We expected that primed lymphocyte test responses might be restricted by histocompatibility at the Ia region. This restriction has affected proliferative responses to soluble antigens in mouse^{15,16} and man¹⁷. In our study, proliferative responses to malignant cells in MLTC-blast-CLC appeared to be limited by tumour site and histology, not by HLA. Three squamous cell carcinomas of the lung (one of which is given in Table 2) showed complete cross-reactivity in that allogeneic SCC also induced significant restimulation while no stimulation was induced by small cell carcinoma or adenocarcinoma of the lung, tumours of the ovary or colon, or blasts from acute myelogenous leukaemia. Similar results were obtained for CLC from nine patients with adenocarcinoma of the breast. Allogeneic tumours induced variable degrees of restimulation but levels of 3H-thymidine incorporation were often comparable with those induced by autologous tumour. The panel of stimulator cells is now being expanded to investigate further the specificity of this primed lymphocyte test response, but the data contrast with the high individual specificity seen in cytotoxicity assays. These results may be explained by HLA restriction of killing to HLA-A, -B or -C (ref. 18) or by individual tumour-associated antigens, whether or not they involve HLA restriction. The question remains as to whether the lymphocyte-stimulating and cytotoxic T-cell target antigens are different18.

We have thus obtained cultured cells having specific antitumour reactivity by adopting approaches which, in our laboratory and in others, have been effective in inducing cultured cells having specificity for alloantigens ¹⁸⁻²⁰. Critical features of these experiments were: (1) the use of MLTC-positive cultures (while cultures could be initiated from MLTC-negative individuals, no specific reactivity was found in these CLC); (2) isolation of blast cells-without this, the cultured cells showed features of polyclonal T-cell activation and high NK activity.

Using these MLTC-blast-CLC, we have demonstrated selective lysis of autologous tumours and restimulation in primed lymphocyte test assays. The nature of the antigens against which these responses were directed is now being investigated. Although there is no evidence that reactivity of specific CLC was directed against HLA-D/DR (failure to lyse autologous monocytes or undergo restimulation by monocytes and lymphocytes), the induction of autoreactivity to other 'normal' antigens, such as organ-specific or differentiation markers, cannot be excluded. We envisage that access to further normal

control tissues should aid the investigation of these reactions. as should the availability of T-lymphocyte clones.

We thank Sheldon Grove for supplying conditioned media under contract YOY DB 00319 with the Naval Medical Research Institute and the Uniformed Services University of Health Science, and Drs R. Zajtchuk, C. Alford, R. Fisher and R. Holt for clinical material. We also thank Dr R.B. Herberman for support and critical review of the manuscript.

Received 16 September 1981, accepted 4 February 1982

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Amplification and rearrangement of *onc* genes in mammalian species

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Related eukaryotic species usually contain the same number of copies per cell of a given 'unique sequence' gene. In the few described exceptions, such as the preproinsulin1,2 and globin genes3-7, one or two additional copies per cell have been found in several related species, suggesting that germ-line amplification occurred millions of years ago in a common progenitor of these species. The transforming (onc) genes of retroviruses are a group of evolutionarily conserved genes for which at least 10 distinct members have been described (for a review see ref. 8). Sequences related to each onc have been identified in all vertebrate species tested, usually as one copy per haploid genome, although the rat has at least two different genes homologous to the onc of the rat-derived Harvey murine sarcoma virus (Ha-MuSV)9. In screening genomic DNA from several rodent species for sequences related to the onc of Ha-MuSV and to the closely related (but distinguishable) onc of Kirsten (Ki) MuSV¹⁰, we have now found evidence for relatively recent amplification of these genes. We report here that Mus pahari apparently contains at least 10 copies of Ha-MuSV-type onc, whereas most other Mus species contain only one or two copies. Similarly, Chinese hamsters (Cricetulus griseus) have about six copies of the Ki-MuSV-type onc compared with only one copy in Syrian hamsters (Mesocricetus auratus).

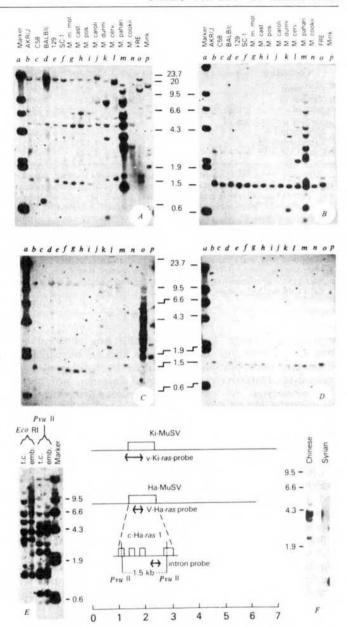
In agreement with recently proposed nomenclature¹¹, the onc of Ha-MuSV has been designated v-Ha-ras, while the two homologous rat cellular genes which have been molecularly cloned are called c-Ha-ras1 and c-Ha-ras2 respectively. (In ref. 9, c-Ha-ras1 is called sarcII and c-Ha-ras2, sarcI. Sarc II

is called c-Ha-ras 1 because it is the most highly conserved form of c-Ha-ras ¹².) Each rat cellular gene is homologous to v-Ha-ras in ~0.9 kilobase (kb)⁹. In c-Ha-ras 2 these homologous sequences are co-linear with v-Ha-ras, while c-Ha-ras 1 has intervening sequences (introns; see Fig. 1). The rat c-Ha-ras 1 gene, v-Ha-ras and v-Ki-ras (the onc of Ki-MuSV), each encode 21,000-molecular weight proteins (p21) which are biologically, immunologically and functionally related 9.10,13-15, although v-Ha-ras and v-Ki-ras DNA probes hybridize in stringent conditions to different restriction enzyme fragments of vertebrate genomic DNAs 10. High levels of virus- or cell-encoded p21s can induce cellular transformation 9.10,13.

To detect and analyse the ras sequences in rodent DNA, EcoRI-digested embryo or tissue culture genomic DNAs from several inbred strains of M. musculus, as well as from other Mus species, rat and mink (a non-rodent), were electrophoresed, transferred to nitrocellulose paper and hybridized to a probe derived from either v-Ha-ras or v-Ki-ras (Fig. 1A, C). The v-Ha-ras probe detected the same c-ras fragments in all M. musculus and in Mus castaneus and Mus poschiavinus (Fig. 1A), while the pattern of c-ras fragments was distinctive for the DNAs from mink, rat, and the other Mus species. The most striking observation, however, was that, in contrast to the relatively few c-ras fragments in the other species, the DNA of a M. pahari cell line (Fig. 1, lane m) contained many fragments which hybridized strongly to the v-Ha-ras probe. We estimate that these fragments represent at least 10 copies per haploid genome. As the v-Ki-ras probe hybridized to only two fragments in M. pahari DNA (Fig. 1C), we conclude that the amplification in M. pahari consisted of c-Ha-ras sequences. (The multiple fragments seen in rat DNA using the v-Ki-ras probe are due, as noted previously10, to the presence of some non-ras viral sequences in the probe; hybridization of cloned human c-Ki-ras DNA12 to rat DNA revealed only four EcoRI fragments; data not shown.) The amplification of the Ha-ras sequences in M. pahari was not a result of in vitro propagation of the cell line, as hybridization of genomic DNA from both the cell line and M. pahari embryos obtained from a different colony gave almost identical results (Fig. 1E). This result also suggests that the amplification is relatively stable in M. pahari. The transcriptional activity of these multiple c-Ha-ras sequences has not yet been determined, but the M. pahari cell line, which is not morphologically transformed, contains low levels of p21 comparable with those found in other normal tissue culture cells from many other vertebrate species16 (data not shown).

As the rat c-Ha-ras1 contains a 1.5 kb PvuII fragment which serves as a useful marker for the gene (see diagram in Fig. 1), PvuII-digested genomic DNAs were hybridized with the v-Haras probe and with a probe derived from an intron of rat c-Ha-ras1 located within the 1.5 kb PvuII fragment (Fig. 1B, D). For all Mus species the intron probe hybridized selectively to a 1.5 kb PvuII fragment which co-migrated with the rat c-Ha-ras1 1.5 kb PvuII fragment, whereas mink DNA did not contain this fragment (Fig. 1D). The intensity of the 1.5 kb fragment was similar in M. pahari and other cell DNAs. Hybridization of the v-Ha-ras probe to the genomic DNAs (Fig. 1B) revealed that M. pahari contained many more PvuII fragments than did the other DNAs. These results strongly suggest that the gene carrying the intervening sequences is highly conserved among the rodents and that most or all of the additional copies of c-Ha-ras in M. pahari do not contain sequences homologous to the intron probe. The data from the non-rodent (mink) DNA also suggest that the intron sequences are less highly conserved than are the exon sequences of c-Haras1.

The amplification of p21-related sequences is not limited to Ha-ras. Genomic DNA from Chinese hamster ovary (CHO) cells digested with EcoRI contained at least eight fragments which hybridized to the v-Ki-ras probe, whereas Syrian hamster DNA contained only one such fragment (Fig. 1F). Similar results have been obtained with BamHI and KpnI digestions



Blot hybridization of genomic DNAs to ras probes. Restriction endonuclease-digested high molecular weight DNAs were electrophoresed in 0.5% agarose slab gels, transferred to nitrocellulose filters²⁶ and hybridized to cloned *ras* DNA labelled with ³²P by nick-translation²⁷. A and B, P-labelled v-Ha-ras hybridized to EcoRI-digested and PvuII-digested cell DNA respectively. C, 32P-v-Ki-ras vs EcoRI-digested cell DNA; D, ³²P-intron of c-Ha-ras vs PvulI-digested cell DNA. E, ³²P-v-Ha-ras vs M. pahari tissue culture (t.c.) and embryo (emb.) DNA. F, ³²P-v-Ki-ras vs EcoRI-digested hamster cell DNA. In A-D, AKR/J, C58, BALB/c and 129 DNAs were isolated from embryos, and other DNAs were isolated from tissue culture cells. M. m. mol, Mus musculus molossinus; M. cast., M. casteneus; M. pos., M. poschiavinus; M. cerv., M. cervicolor; FRE, Fischer rat embryo cells. Marker was ³²P-labelled HindIII-digested λ DNA. Fragment sizes are in kb. The M, pahari adult tail tissue culture line²⁸ (t.c. in E) was established in 1979 from a mouse provided by T. C. Hsu; the embryo DNA (emb.) was isolated from a mouse provided in 1981 by Richard Sage. In F, Chinese = CHO cells²⁹; Syrian = Syrian baby hamster kidney cells³⁰. In A, the probe has hybridized to four fragments of M. musculus DNA: 23, 7, 5 and 1.5 kb. In other experiments the v-Ha-ras probe consistently hybridized to the 23 kb fragment, but the hybridization to the other fragments was less consistent. The 7 and 5 kb fragments probably represent uncharacterized sequences homologous to c-Ha-ras. The 1.5 kb fragment probably represents c-Ki-ras sequences, as the v-Ki-ras probe consistently hybridizes to a 1.5 kb fragment (B). In the diagram, the relative locations of the ras sequences in the linear viral DNAs are indicated by a rectangle. The locations of sequences in the rat c-Ha-ras1 gene homologous to v-Ha-ras are shown by boxes; the spaces between these boxes represent introns. The arrow beneath each DNA indicates the location of sequences in each probe: the v-Ki-ras probe is the 0.6-kb SstII/HincII fragment of clone HiHi-3 (ref. 10); the v-Ha-ras probe is clone BS-9 (ref. 9), and the intron probe is a 0.3 kb HincII fragment obtained by electroelution from a clone of c-Ha-ras1 in pBR322 (ref. 9).

(data not shown). As in the case of the M. pahari embryos, DNA from Chinese and Syrian hamster embryos resembled DNA from the cell lines of the respective species (data not shown).

Since closely related species contain only one or two copies of these amplified gene sequences, the ras copy number has probably increased relatively recently in M. pahari and in Chinese hamsters. The ras amplification therefore differs from other amplified unique sequence genes, such as globin and preproinsulin, in its high copy number and recent origin, from that of described orphons¹⁷ in its stability and in its affecting unique sequence genes, and from the transient nonchromosomal amplification of ribosomal genes during amphibian oogenesis1

The structure of the amplified ras sequences remains to be established. The lack of homology to the intron of c-Ha-ras1 indicates that the amplified Ha-ras genes differ in structure from the c-Ha-ras gene with introns. The results suggest that these sequences may represent genes which lack introns entirely, as was the case in the rat c-Ha-ras2 gene, although the data reported here are compatible with other structures. If the amplified sequences lack introns, their structure would also resemble that of c-mos, the onc which gave rise to the transforming sequences of Moloney murine sarcoma virus¹⁹, and of an α -globin pseudogene which is present in mice and other species^{3,4,7}. The analogy of c-mos suggests that, unlike pseudogenes, the amplified ras sequences might encode a biologically active p21.

It is not known what conditions and molecular mechanisms led to the amplification of the ras genes, whether the amplified genes are clustered or dispersed, or what the biological consequences of this amplification may be. Analysis of the sensitivity of the M. pahari and Chinese hamster cells to the biological activity of p21, the distribution of the ras sequences within the cell genome, the structure of the amplified genes and their biological potential should, however, yield significant insights into these amplified sequences.

The amplification is probably not due to a general tendency of these animals to amplify genes as in M. pahari neither the closely related c-Ki-ras sequences nor the β -globin sequences were amplified (A. Leder, personal communication). The amplification might represent a genetic response to a noninfectious environmental agent, such as a chemical. Marked amplification of the dihydrofolate reductase (DHFR) gene has been induced in tissue culture cells in response to methotrexate; in cells with stable methotrexate resistance, the amplified DHFR genes are chromosomal and clustered²⁰. Alternatively, because transforming retroviruses arise by transduction of cellular onc genes⁸, the amplification might have arisen by this mechanism through putative endogenous retrovirus-like sequences or even through horizontal viral infection. In this model the ras sequences would be dispersed, as the termini of retroviral genomes resemble those of transposons and they integrate at multiple sites^{21,22}. In addition, endogenous retroviral genomes are usually found in multiple copies and may be absent from closely related species²³. This mechanism may also be relevant to the mouse α -globin pseudogene which lacks introns; it is not linked to the α -globin cluster²⁴ and has recently been found to be flanked by A-particle genes25, which are retrovirus-like genetic elements. Finally, the amplification may represent a response to a functional lesion in p21 or to a relative resistance of these cells to the protein. However, we presume that the animals tolerate the amplification because these amplified genes are not ordinarily expressed, as suggested by the normal p21 levels in the M. pahari cell line, but firm conclusions must await further study of the expression and biological potential of these genes.

Received 23 September 1981, accepted 19 January 1982

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DNA sequence homology and chromosomal deletion at a site of SV40 DNA integration

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The structure of simian virus 40 (SV40) DNA insertions is different from those of retrovirus proviruses and movable genetic elements. No single DNA sequence in either the cell or the SV40 genome serves as an obligatory site of SV40 integrative recombination1-9, and SV40 DNA insertions are not bordered by the repeat structures characteristic of transposons and retrovirus proviruses¹⁰⁻¹⁵. Integration of SV40 could involve the matching of short stretches of homologous sequences present in the otherwise heterologous SV40 and cellular genomes. To explore this possibility, I have now sequenced a rat DNA fragment that contains an unoccupied site of SV40 DNA integration. Comparison of this sequence with that of SV40 and that at the rat-SV40 recombinant junction allowed two conclusions: first, SV40 DNA became linked to rat DNA at a point where the two genomes shared 5 base pairs (bp) of DNA sequence homology; and second, a rearrangement of the rat genome, probably a deletion of at least 3 kilobases (kb), occurred at the site of SV40 integration.

The SVRE9 cells, chosen for this study, were Fisher rat embryo fibroblasts that were transformed by infection with SV40¹⁶. In previous work, the single viral DNA insertion in those cells was cloned and the sequences at the rat-SV40 junctions were determined8. Figure 1 shows the structures of the cloned SV40 DNA insertion, Sst9, and the subcloned SV40rat junction fragments, 9M and 9L.

To test their suitability as probes with which to isolate a rat DNA fragment containing the unoccupied site of SV40 integration, junction fragments 9M and 9L were radiolabelled and hybridized to Southern¹⁷ blots of restriction endonucleasedigested rat DNA. 9M DNA was unsuitable in that it hybridized to many size species of endo: R: EcoRI-digested rat DNA, indicating that this junction fragment contained a sequence that was repeated many times in the rat genome (not shown). By contrast, 9L DNA hybridized to a single rat DNA fragment and was apparently free of repeated DNA sequences (see Fig. 2).

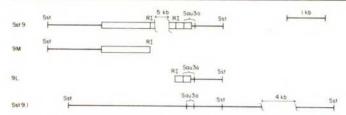


Fig. 1 Physical maps of cloned DNA fragments. Open bars represent SV40 DNA, lines represent cellular DNA. Sst9 was cloned from a λ phage library, 9M and 9L were cloned in plasmid pBR322. Cloning and analysis of this DNA were as previously described. Clone Sst9.1 was isolated as described in the text and mapped by restriction endonuclease analysis and by hybridization using 9L DNA as a probe. The DNA fragments are drawn to align homologous sequences.

Figure 2 shows that SVRE9 cells contained two DNA fragments with homology to 9L DNA; one fragment co-migrated with the fragment bearing the SV40 DNA insertion, and a second fragment co-migrated with the normal rat DNA fragment homologous to 9L DNA. These data are most simply interpreted to indicate that SVRE9 cells are haploid for their single SV40 DNA insertion; the two SVRE9 DNA fragments detected by the 9L DNA probe probably originated from a pair of homologous chromosomes, one carrying the SV40 DNA insertion and the other containing a copy of the unoccupied SV40 integration site.

The putative unoccupied integration site was isolated from a library of endo: R: SstI-digested SVRE9 DNA using procedures previously described^{8,9}. The chimaeric λ phage Sst9.1 (shown in Fig. 1) contained two SstI fragments 4 kb and 6 kb in size. Analysis by restriction endonuclease digestion and blot hybridization (Fig. 3 and other data not shown) mapped the 9L homology to one end of the 4 kb fragment of Sst9.1 DNA, as indicated in Fig. 1.

To determine whether any of the DNA sequences flanking the 9M junction were present in Sst9.1 DNA, radiolabelled 9M DNA was hybridized to Southern blots of SstI-digested Sst9.1 DNA. As shown in Fig. 3, none of the DNA in Sst9.1 was homologous to 9M DNA. The absence of homology between 9M DNA and Sst9.1 DNA is most simply explained by deletion of at least 3 kb of the rat genome at the site of SV40-rat DNA linkage in the SVRE9 progenitor cell.

Analysis of Sst9.1 DNA by restriction endonuclease digestion and blot hybridization localized the SV40 integration site to a 200-bp endo: R: Sau3a fragment (see Fig. 1). The nucleotide sequence of this DNA fragment was determined by the protocol of Maxam and Gilbert¹⁸ and the results are shown in Fig. 4. Comparison of the Sst9.1 DNA sequence with those of SV40 and the 9L junction revealed that SV40 DNA and rat DNA shared a 5-bp sequence homology at a point coincident with the rat-SV40 junction in 9L DNA.

This is the first direct demonstration that DNA sequence homology may mediate integrative recombination between SV40 DNA and the cellular chromosome. Two other studies indicate that animal cells have the ability to recombine heterologous DNA molecules through short sequence homologies. Gutai¹⁹ found two cases of 3-bp homologies at virus-virus recombinant joints in naturally arising variants of SV40. M. Botchan and co-workers (personal communication) have found evidence that short sequence homologies are involved in the imprecise excision of a SV40 DNA insertion.

The DNA sequence of the unoccupied SV40 integration site shown in Fig. 4 is striking in two ways. It is repetitive, containing 12 T \cdot G pairs in tandem, and it is composed almost entirely of G and T. Thus, this DNA chain is simple in both its sequence arrangement and its base composition. The T \cdot G repeat does not extend past the nucleotides shown in Fig. 4. In addition to the 34 bases shown, the sequence 80 nucleotides to the left and 25 nucleotides to the right were determined. The T \cdot G repeat is remarkably similar to the 'simple sequence' DNA

reported by Slightom et al.20 to be present in the second introns of human y-globin genes. Comparison of sequence similarities and differences between allelic $^{A}\gamma$ genes and non-allelic $^{G}\gamma$ and A y genes led these authors to suggest that this simple sequence region was a hot spot for DNA exchanges leading to gene conversion. As the non-allelic human γ -globin genes are $\sim 97\%$ homologous, simple sequence DNA is postulated to provide more than sequence homologies through which to initiate strand exchanges between genes. Rather, simple sequence DNA seems to act specifically by virtue of its unusual sequence, at least in the case of human y-globin genes. Gene conversion has been suggested as a mechanism by which multi-gene families may conserve the identity of their members²¹, and simple sequence DNA may be of general importance in the gene conversion processes. The T · G repeat found in rat DNA may thus be one of many such repeats in the cellular genome.

These considerations bear on the evaluation of the possible contribution of the simple sequence DNA in Fig. 4 to the integration of SV40 DNA. There are two obvious possibilities: (1) simple sequence DNA may be a target for specialized recombination enzymes designed to accomplish gene conversions; these enzymes may act to integrate SV40 DNA. (2) Simple sequence DNA may be present in many copies, thereby increasing the probability of integration in such a sequence.

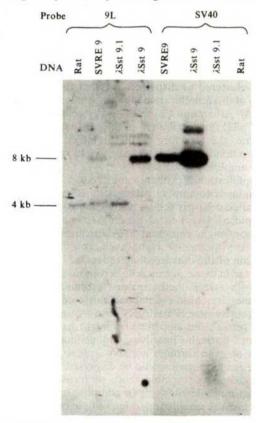


Fig. 2 Detection and sizing of rat DNA fragments containing sequences homologous either to SV40 DNA or to DNA flanking the 9L junction of the SV40 DNA integrated in SVRE9 cells. DNA (10 µg) from either Fisher rat embryo cells or SVRE9 cells were digested with SsfI and electrophoresed through 1% agarose in 36 mM Tris, 30 mM NaH2PO4, 1 mM EDTA pH 7.5. Chimaeric λ phage, Sst9 and Sst9.1, bearing the DNA fragments shown in Fig. 1, were mixed with calf thymus DNA and cut with SsI. 1×10^{-5} µg of each phage DNA was run in parallel with rat cell μg of each phage DNA was run in parallel with rat cell DNA samples. By methods previously described1, gels were blotted on to nitrocellulose which was then hybridized to either 9L DNA in pBR322 or SV40 DNA, each of which had been labelled to high specific activity by nick translation. DNA homologous to radioactive probes was detected by autoradiography. Faint bands in A Sst9 and A Sst.9.1 tracks in the 9L panel are due to weak hybridization between pBR322 and λ arms. Minor bands in λ Sst9 track in SV40 panel are partially digested chimaeric phage DNA.

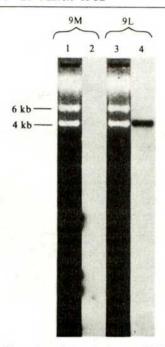


Fig. 3 Lack of homology between the rat DNA sequences in clone Sst9.1 and the rat DNA flanking the 9M junction of the SV40 DNA integrated in SVRE9 cells. A λ phage-bearing Sst9.1 DNA (Fig. 1) was digested with Ssf1 and electrophoresed on a 0.7% agarose gel. The gel was stained with ethidium bromide, photographed under UV illumination, blotted and the blot hybridized to one of two radiolabelled probes: 9M DNA, lanes 1 and 2; or 9L DNA, lanes 3 and 4. Lanes 1 and 3 are ethidium bromide-stained gels. Lanes 2 and 4 are autoradiographs of the same gels, blotted and hybridized as described elsewhere 9 M DNA did not hybridize to either of the cell DNA fragments in Sst9.1, while 9L DNA hybridized strongly to the 4-kb DNA fragment present in Sst9.1

Although both possibilities are attractive, there is no reason to believe that simple sequence DNA is necessarily involved in integration of SV40 DNA. The sequence labelled 'SVRE9' in Fig. 4 shows that homologous recombination of SV40 DNA with a portion of the repetitive rat DNA sequence generated a SV40-cell junction in which the terminal viral sequence, TGTGT, is repeated in the adjacent cellular DNA. In previous analysis of the nucleotide sequences at other cell-virus junctions, I found a similar repeated structure, composed of C and T, present at one junction of the SV40 DNA insertion present in SVRE17 cells8. However, of the six cell-virus recombinant junctions compared in that study, only two, one junction of SVRE9 and one of the SVRE17 DNA insertion, showed repeat structures. In addition, none of the three viral DNA insertions compared in that study showed repeat structures at both ends of an integrated SV40 DNA. Therefore, although it is not uncommon, repeated, simple sequence DNA is not a requisite feature of DNA adjacent to SV40 DNA insertions. The data in Fig. 4 suggest that the presence of a terminal repeat structure at a cell-virus junction may be indicative of homologous integration into a repeated cellular sequence, but the lack of such structures does not rule out homologous recombination with non-simple sequence DNA.

Integration of SV40 DNA may exploit the great sequence complexity of the mammalian genome. Any 5-bp sequence present in the SV40 genome would be expected to occur of the order of 10⁶ times in the unique sequence component of a rat cell genome. Perhaps integration is initiated when a random short sequence in the viral genome finds its match in chromosomal DNA. Integration might be completed by a second, independent ligation reaction at a different chromosomal location. Such a mechanism, of course, would generate chromosomal deletions at integration sites. Models predicting chromosomal deletions have been proposed previously (ref. 6 and

Botchan in ref. 22) and all evidence to date indicates that deletion of substantial amounts of the cellular chromosome is a consequence of SV40 DNA integration. The SVRE9 chromosome suffered a loss of at least 3 kbp of DNA, and there has been one other report consistent with chromosomal deletion at a SV40 DNA integration site. Analysis of a third SV40 DNA insertion, in SVRE17 cells, also indicates a loss of chromosomal sequences at the site of SV40 DNA integration (J.R.S., unpublished data). Three examples do not compel the conclusion that SV40 DNA integration is always accompanied by chromosomal deletions, but no exceptions have yet been found.

The finding of short sequence homology at a SV40 DNA integration site implies that DNA sequence homology might direct an exogenous DNA to integrate homologously into a unique chromosomal copy of itself. This is the case in yeast cells; exogenous yeast genes recombine homologously with their chromosomal counterparts²³. However, there is reason to doubt the ability of animal cells to match an exogenous DNA sequence efficiently with a single chromosomal copy of the same sequence. Botchan et al.²⁴ reported that when SV40-transformed rat cells were superinfected with SV40, the newly introduced viral genomes did not integrate into pre-existing SV40 DNA insertions. The difference between yeast cells and rodent cells may simply reflect the larger size of the mammalian genome. Exogenous SV40 DNA may not be able to find a single chromosomal copy of itself, and instead be integrated through the mediation of a short, but highly frequent, random sequence homology. Whatever the reason, integration of exogenous DNA into a unique homologous chromosomal site of an animal cell genome seems to be a low probability event.

SV40 integration is the best studied case of incorporation of a foreign DNA molecule into the cellular genome. Many SV40 DNA insertions have been analysed by restriction endonuclease mapping¹⁻⁷ and six SV40-cell recombinant junctions have been sequenced^{8,9}. These studies have established that, unlike retroviruses and movable genetic elements, integration of SV40 DNA insertions does not involve specific DNA sequences in either the viral or cellular genomes, nor does it involve generation of short duplications of host sequence at the point of integration. Although analysis of more unoccupied sites of SV40 DNA insertion will be needed to understand fully the role of base pairing in SV40 integration, the data presented above demonstrate that cells are able to use a short sequence homology to integrate an otherwise heterologous SV40 DNA molecule. This example of homologous recombination suggests an additional difference between integration of SV40 DNA and retroviruses and transposition of movable elements.

Understanding cellular recombination systems that foster integration of exogenous DNA is important in assessing the practicability of schemes that propose the introduction of therapeutic genetic material into the genomes of defective cells. Integration of SV40 DNA probably reflects the action of a cellular recombination system that can incorporate any foreign



Fig. 4 DNA sequences at the SV40 integration site. The sequence in Sst9.1 DNA homologous to the rat DNA immediately adjacent to SV40 DNA in clone 9L was shown, by hybridization experiments, to lie in a 200-bp Sau3a fragment (see Fig. 1). The nucleotide sequence of 140 bp of this DNA was determined by the method of Maxam and Gilbert 18, and the relevant portion of the sequence is labelled 'Rat'. Also shown is the sequence of SV40 DNA as reported by Reddy et al. 25, and the sequence at the 9L junction of the SV40 DNA insertion in SVRE9 cells, as previously determined by J.R.S. Vertical lines designate homologous nucleotides. Comparison shows that SV40 DNA and rat DNA share 5 bp of homology at a point coincident with the SV40-rat junction in the cloned 9L junction fragment.

DNA into the cellular genome, and analysis of SV40 DNA insertions may predict the fate of other exogenous DNAs. Although cells may incorporate exogenous DNA efficiently, that DNA is unlikely to integrate into a homologous single-copy sequence in chromosomal DNA. Instead, foreign DNA might be expected to integrate randomly through a mechanism that uses short sequence homologies, and generates chromosomal deletions

I thank Laurel Garbarini for technical assistance, Patti Barkley, Mike Ockler and Eric Lockhart for help in preparing the manuscript, Joe Sambrook for suggesting improvements in the manuscript and Mike Botchan for drawing my attention to the potential significance of simple sequence DNA. This work was supported by PHS CA-13106 and NCI grant CA-06460.

Received 4 November 1981; accepted 5 February 1982.

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Influenza virus RNA is synthesized at fixed sites in the nucleus

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We have recently shown that cellular RNA is synthesized at a sub-nuclear structure, the nuclear cage1, which contains proteins also found in other structures called variously the nuclear pore complex, lamina, envelope and matrix²⁻⁶. Is the RNA of an exogenous virus also synthesized at the cage? We chose to study influenza virus as it is unusual in its requirement for a host cell nucleus even though there are no cellular counterparts to the transcription of the infecting negative strands of genomic influenza RNA, nor to the replication of the resulting positive RNA strands to form new virion RNA. The cellular sites of these processes have not yet been definitively demonstrated⁷ We now show that nascent viral transcripts are closely associated with the cage and we conclude not only that transcription and replication of viral RNA are nuclear, but also that they occur at fixed sites in the nucleus.

Several observations suggest a nuclear involvement during the transcription and replication of influenza virus⁷⁻¹⁰. These include nuclear labelling in autoradiographs, inhibition of viral production by actinomycin D and α -amanitin—both inhibitors of nuclear RNA polymerase II—and the splicing and methylation of viral mRNA. However, attempts to demonstrate directly the site of viral RNA synthesis by pulse-labelling and cell fractionation have yielded variable results 11-13. We believe that this variability is caused by long pulse-labels (≥30 min) and nucleolytic action during cell fractionation, both of which permit

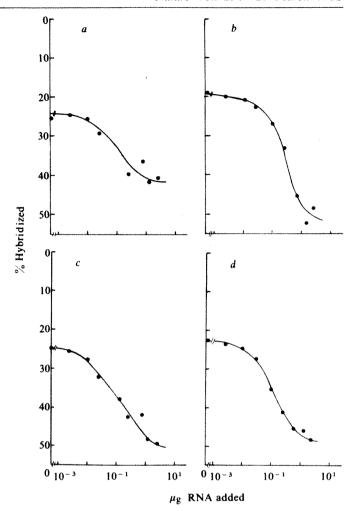


Fig. 1 The proportion of viral transcripts in pulse-labelled RNA from infected cells. Cells were infected and pulse-labelled (2.5 min) with 3 H-uridine after 2.5 h (a, b) or 4.5 h (c, d). Nucleoids were then isolated as described in Table 1 legend, pulse-labelled RNA was purified and hybridized with various amounts of virus-specific RNA obtained from purified virions (vRNA) or by extracting polyadenylated RNA from the cytoplasm of infected cells 7 h post-infection (mRNA)¹⁸. (Such a mRNA preparation is impure and contains some cellular RNA, and perhaps low levels of vRNA. However, the cellular impurities have no complements and so cannot form hybrids.) Less than 5% of pulse-labelled RNA from uninfected cells hybridized with these virus-specific RNAs. The per cent forming a hybrid resistant to S_1 nuclease was determined S_1 . Hybridizations (10 days) were carried out in a volume of 5 µl containing at least 5,000 c.p.m. of pulse-labelled RNA (~2.5 μg of total nucleoid RNA), 2.5 μg tRNA and up to 2.5 μ g mRNA (a, c) or vRNA (b, d). These conditions give a maximum C_0t of 10^3 mol s l

transfer between cell compartments. We have therefore used very short pulses (2.5 min) and a cell fractionation procedure which minimizes nucleolytic degradation.

Embryonic chick fibroblasts were lysed in a non-ionic detergent, 2 M salt and a chelating agent. The resulting nucleoids were sedimented free of cytoplasmic material and contained naked histone-free DNA packaged within a flexible cage of RNA and protein¹⁴⁻¹⁷. Their DNA, which is looped by attachment to the cage, is supercoiled^{14,15}, indicating that nucleolytic degradation has been suppressed during isolation.

When mock-infected fibroblasts are incubated with ³Huridine for 2.5 min, 96% of the cellular radioactivity which is insoluble in trichloroacetic acid subsequently co-sediments with nucleoids (Table 1). Nearly all the label also co-sediments with nucleoids made from infected fibroblasts pulsed at early (2.5 h) or late (4.5 h) times during infection. We determined what proportion of nascent RNA in infected cells was virally coded

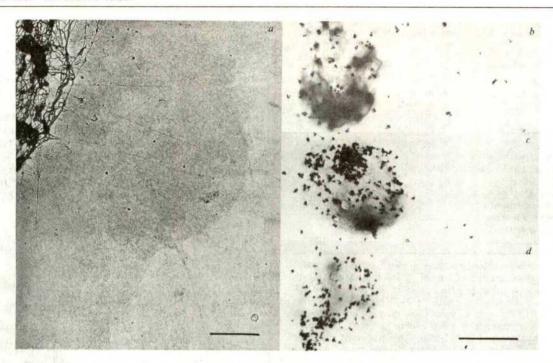


Fig. 2 Electron micrographs of nucleoids spread by Kleinschmidt's procedure. a, A typical spread illustrating part of the cage and skirt of tangled DNA fibres, which extend to the edge of the field. b-d, Autoradiographs of spreads 19 after labelling uninfected fibroblasts for b, 24 h with 3 H-thymidine $(0.01 \,\mu\text{Ci ml}^{-1}; 58 \,\text{Ci mmol}^{-1})$ or for c, 2.5 min with $[5-6-^{3}\text{H}]$ uridine and $(2,5'-8-^{3}\text{H}]$ adenosine (both at $100 \,\mu\text{Ci ml}^{-1}$ and $\sim 40 \,\text{Ci mmol}^{-1})$; or d, infected fibroblasts $4.5 \,\text{h}$ post infection for 2.5 min with ^{3}H -uridine and ^{3}H -adenosine as in c. So that silver grains can be easily counted in b-d, DNA is shadowed but not stained and can be seen spread to the edge of the field only at higher magnifications. In b, grains lie over both cage and skirt, whereas in c and d they lie predominantly over the cage. Conditions for infection are described in Table 1 legend. The bars in a and d represent 5 and 2.5 μ m respectively. b-d Are at the same magnification.

by isolating pulse-labelled nucleoid RNA and hybridizing it with an excess of unlabelled virion (v) RNA or mRNA (Fig. 1), which hybridize with strands of positive and negative polarity respectively18. In the absence of added vRNA or mRNA, <1% of the pulse-labelled RNA from mock-infected cells selfanneals, reflecting the asymmetric transcription of cellular DNA. In contrast, about 25% from infected cells self-anneals, reflecting the synthesis of both positive and negative strands. In the presence of an excess of either vRNA or mRNA, 45-50% of the pulse-labelled RNA hybridizes, whereas ~70% hybridizes in the presence of an excess of both vRNA and mRNA (results not shown). We cannot give a precise estimate of the proportion of nascent transcripts which are viral, as our mRNA probe is impure (see Fig. 1 legend) and the significance of the self-annealed values is difficult to assess. Nonetheless, it is clear that positive and negative viral transcripts constitute a significant proportion (>50%) of the pulse-labelled material.

The pulse-label might co-sediment with cages, either because it is specifically attached or because it is tangled in the high concentration of nuclear DNA. We tested the latter possibility by finding out whether RNA could be released on detaching DNA from the cages. Cells were labelled with ¹⁴C-thymidine for 24 h to label their DNA uniformly, then infected and subsequently pulsed with ³H-uridine. Nucleoids were isolated, incubated with the restriction endonuclease, *EcoRI*, the cages were filtered free of detached DNA and the amounts of the two labels remaining associated with them determined ¹⁹ (Table 1). When >75% of the ¹⁴C (that is, DNA) was detached, <1% of the ³H (RNA) was lost, showing that the pulse-labelled RNA cannot be detached with DNA from the cages.

A second experiment confirms that nascent RNA is not simply entangled in DNA or the cage. When nucleoids are spread on an air-aqueous interface, their DNA, initially confined within the cage, spreads out from the cage to form a surrounding skirt of tangled DNA fibres¹⁷ (Fig. 2a). Cages from chick fibroblasts are not as robust as those of the HeLa nucleoids that we have studied extensively, so the DNA is less protected

from shear. As a result, more is broken and therefore appears relaxed after spreading. The spread DNA is less dense, reflecting the lower DNA content of the diploid chick nucleus. The distribution of DNA in skirt and cage was obtained by reference to autoradiographs of spreads prepared from cells containing uniformly labelled DNA (Fig. 2b); 53% (average of 15 spreads) of the DNA is outside the cage. However, when cells—whether infected or not—are pulse-labelled with ³H-uridine for 2.5 min, >85% of the grains lie over the cage in each of 10 spreads selected at random (Fig. 2c, d). The proportion of grains lying over the nucleolus is reduced in spreads from infected cells (see Fig. 2c, d) late in infection, reflecting the reduced percentage of ribosomal (cellular) RNA synthesis in the total. The nascent RNA—largely viral—is unable to escape with the DNA from the cage.

A trivial explanation consistent with these results is that nascent viral RNA, synthesized throughout the nucleus, might stick nonspecifically when nucleoids are prepared. Our earlier study1 showed that nascent RNA in uninfected HeLa cells was specifically attached at the 5' end, whereas added RNA, or RNA synthesized in vitro, was either unattached or was nonspecifically associated with cages but could be detached from them by EcoRI or spreading. Using identical conditions we have shown here that nascent viral RNA is inseparable from cages. Three further controls make a nonspecific association even less likely. First, when pulse-labelled RNA from infected cells is purified and mixed with unlabelled infected cells immediately before lysis, <3% of the pure RNA co-sediments with the nucleoids, indicating that the added RNA has little affinity for cages. Second, we have confirmed earlier results9 which showed by autoradiography that all the pulse-labelled RNA in infected cells was nuclear. After a 2-h chase, the total number of grains over the cell was reduced, reflecting turnover: half lay over the nucleus, the remainder being cytoplasmic (results, not shown, of experiments using conditions described in Table 1). When these cells are lysed in 2 M salt, only 40% of the label now co-sediments with the nucleoids (Table 1). Although

Table 1 Pulse-labelled RNA is closely associated with cages

	% Label co-sedimenting with nucleoids	associa cages incul	remaining ted with after pation
	³H	14C	³ H
Mock-infected cells (2.5 min pulse)	96	17	100
Mock-infected cells (2.5 min pulse, 2 h chase)	58	*******	_
Cells 2.5 h post-infection (2.5 min pulse) Cells 2.5 h post-infection	93	25	99
(2.5 min pulse, 2 h chase)	40		_
Cells 4.5 h post-infection (2.5 min pulse)	92	20	100

Primary chick embryo fibroblasts were grown³⁰ for 24 h in [Me
14C]thymidine (0.01 µCi ml⁻¹; 58 Ci mmol⁻¹), infected (MOI 100:1) where appropriate with influenza virus (influenza A/PR8/34) and 2.5 or 4.5 h later pulse-labelled with [5,6-3H]uridine (2.5 min; 100 μCi ml⁻¹; ~40 Ci mmol⁻¹). In some cases the pulse was followed by a 2-h chase in the presence of 1 mM uridine. 2.5×10^6 cells were removed, washed and lysed in 1.95 M NaCl on step gradients containing 1.95 M NaCl, spun (Beckman SW50.1 rotor; 15,000 r.p.m.; 45 min) to sediment the nucleoids on to the step and the percentage of ³H co-sedimenting with the nucleoids was determined ^{15,16}. Nucleoids were collected, diluted to 0.2 M NaCl, incubated with EcoRI and the proportion of labels remaining associated with cages was determined after filtration^{1,19}. A control experiment showed that >97% of the ³H but filtration^{1,19}. A control experiment showed that >97% of the ³H but none of the ¹⁴C could be detached from cages by ribonuclease.

nucleoids contain some elements of the cytoskeleton (that is, actin and intermediate filaments), the cytoplasmic RNA does not co-sediment with the nucleoids.

For the third control we studied Chandipura virus, a rhabdovirus with a single genomic RNA strand of negative polarity and whose reproduction is cytoplasmic²⁰. Plaque-purified virus²¹ was grown in chick fibroblasts (conditions and multiplicity of infection similar to those described in Table 1 legend so that cytopathic effects were visible after 7 h) in the presence of a concentration of actinomycin D (0.5 µg ml⁻¹ added 1 h post-infection) which suppresses nuclear RNA synthesis by >95%. When, at 4 h post-infection, cells were labelled for 10 min with ${}^{3}\text{H}$ -uridine (50 $\mu\text{Ci ml}^{-1}$), about 3.7 times more label was incorporated into infected cells than into mockinfected controls; thus, 73% of the labelled transcripts were viral, the remainder being the residual host transcripts. When such cells are lysed and spun, only 30% of the incorporated label co-sedimented with nucleoids; 70% was cytoplasmic and remained at the top of the gradient. Unlike nascent influenza virus transcripts, the transcripts of Chandipura virus are not associated with cages.

We have demonstrated that at early and late times during infection with influenza virus, at least 50% of the nascent transcripts in infected cells are viral and all are attached to the nuclear cage. They remain attached in the detergent and 2 M NaCl used to prepare nucleoids. We might have expected little host involvement in RNA-dependent RNA synthesis, especially as the infecting virus contains a polymerase which is capable of synthesizing its own mRNA in vitro 22,23. It is perhaps not surprising that nascent transcripts of positive polarity are cage associated because they are covalently coupled at their 5' ends to host-encoded sequences^{24,25}, which we have shown are themselves attached1. Attachment of nascent transcripts of negative polarity, which do not contain any host-encoded sequences, is more surprising. However, a complex picture of processing of nuclear transcripts is now emerging. Transcripts are attached to the nuclear cage as they are generated and subsequently as heterogeneous nuclear RNA²⁶⁻²⁸; later they are attached to the cytoskeleton as cytoplasmic message²⁹. (This cytoskeletal attachment is unstable in high salt concentrations.) There are

obvious practical advantages in tying a long RNA molecule to a larger structure during such a complex series of events as synthesis, capping, methylation, splicing, polyadenylation and translocation to the cytoplasm. There are additional problems associated with the regulated transcription and replication of the eight influenza viral segments and attachment could provide a structural basis for this. Therefore, attachment may play a central part in these processes and any viral nucleic acid subverting some, or all, of them, must also become attached.

We thank Professor H. Harris, Professor G. G. Brownlee and Dr B. S. Cox for their encouragement, Dr J. S. Porterfield and Professor D. H. L. Bishop for their help with Chandipura virus, and the Cancer Research Campaign, MRC and SRC for support. Wild-type Chandipura virus was from Dr C. R. Pringle.

Received 11 August 1981, accepted 16 February 1982

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Cytogenetic location and expression of collagen-like genes in *Drosophila*

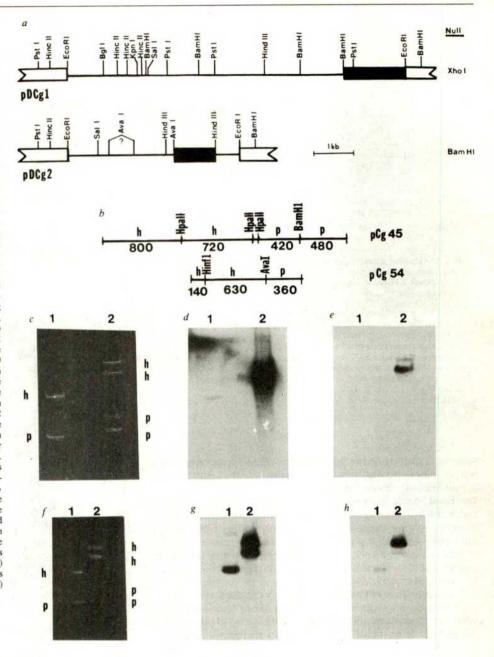
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Much of the present interest in vertebrate collagens stems from the important part which these extracellular, structural proteins play in developmental processes and tissue organization as well as from their complex gene structure. So far the only vertebrate collagen genes examined encode the constituent polypeptide (pro α) chains of type I procollagen, that is, the pro $\alpha 2(I)$ genes from chicken^{1,2} and sheep³, and the pro $\alpha 1(I)$ gene from mouse⁴. Recently, we have isolated several collagen-like genomic DNA clones from Drosophila melanogaster5. In addition to providing data on the evolutionary history of this gene family, studying Drosophila has distinct advantages for cytogenetic localization of genes and for defining the functional roles of individual collagens by the application of genetic techniques. Here we compare the hybridization patterns, cytogenetic localization and expression of two of the Drosophila clones, DCg1 and DCg2. Although they are cytogenetically unlinked, they share similar developmental RNA profiles.

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Fig. 1 Radioactive probes constructed from the Drosophila sequences DCg1 and DCg2 were hybridized to restriction digests of chicken collagen cDNA sequences to determine the regions of homology between the Drosophila chicken sequences. a, Restriction maps of DCg1 and DCg2. Open boxes represent pBR322 vector sequences flanking the inserted Drosophila DNA and solid boxes represent regions of Drosophila DNA that cross-hybridize to the chicken clone, pCg45. Radioactive probes representing the solid regions were made by ³²P nick-translation26 of the following restriction fragments purified from agarose gels by electroelution: pDCg1, 1.5-kb EcoRI/BamHI frag-ment; pDCg2, 1.2-kb internal HindIII fragment. b, Digestion with the appropriate restric tion enzymes divides the chick collagen cDNA insert sequences into discrete fragments that encode either the Gly-X-Y repeat involved in triple helix formation (h) or the carboxyterminal propertide region (p) of the pro α -chain^{6,7}. The pro α 2 clone (pCg45) can be cut with HpaII and BamHI to produce two fragments of 800 and 720 base pairs (bp) encoding regions participating in triple helix formation, and two smaller fragments of 480 and 420 bp representing the propeptide region (see lane 2 of c and f). The pro $\alpha 1$ clone (pCg54) can be cut by AvaI and HinfI into fragments of 630 and 140 bp involved in triple helix formation and a fragment (360 bp) encoding propeptide sequences (see lane 1 of c and f). c-h, The Drosophila probes were hybridized to Southern blots of the restricted chick pro α 1 and pro α 2 collagen cDNA sequences to determine whether the shared homology was localized in areas encoding the triple-helix forming (h) or propeptide (p) regions of the chick sequence. c-e, The DCg2 probe was hybridized to filters in a 50% formamide, 5×SSC hybridization solution26, and washed at 50 °C in 0.5 × SSC, 0.1% SDS; a long exposure (d) and shorter exposure (e) of the autoradiograph are shown. f-h, The DCg1 probe was hybridized as above and washed first at 50 °C in 0.5 × SSC (g) and then at 50 °C in 0.1 × SSC (h). The DCg2 probe hybridizes preferentially to one of the fragments (720 bp) encoding the triple-helix forming (h) region of Cg45 while the DCg1 probe hybridizes most strongly to the other fragment (800 bp) that encodes the triple helix-forming region.



The differences in hybridization of each Drosophila clone to two chicken collagen cDNA clones suggests that the Drosophila sequences represent different members of a family of related collagen-like genes. The isolation of these Drosophila clones was based on sequence homology with a heterologous hybridization probe, the cDNA clone pCg45, encoding half of the chicken pro \(\alpha 2(I) \) chain⁶. As shown in the restriction maps for the subclones pDCg1 and pDCg2 (Fig. 1a), the region of homology to Cg45 extends over only a portion of each clone. Hybridization of Cg45 to these designated subfragments is limited to the region of the cDNA clone encoding the Gly-X-Y sequences that participate in triple helix formation (the α -chain domain) (Fig. 1c-e). Each of the two Drosophila clones hybridizes preferentially to a different section of the chicken cDNA clone encoding the Gly-X-Y repeat (Fig. 1c-h). In addition, DCg1 shares more homology than DCg2 with the α-chain domain specified by a chicken pro a1(I) cDNA clone7 (Fig. 1c-h). Although the number of genetically distinct collagen chains in Drosophila is unknown, there is biochemical evidence in other invertebrates for multiple chain types⁸⁻¹⁵. Therefore the differences in hybridization of the Drosophila clones to the chicken pro $\alpha 1(I)$ and pro $\alpha 2(I)$ cDNA clones suggest that the

former represent structurally distinct collagen-like molecules. However, if the two sequences use different codons preferentially, as observed for other collagen genes^{4,16}, they may simply represent non-allelic copies of a gene encoding one collagen-like protein. DNA sequence analysis of DCg1 indicates that it may encode a segment of a non-fibrous collagen such as a basement membrane or cuticle collagen, or a novel collagenous protein⁵.

At moderately low stringency, the genomic hybridization patterns defined for *Drosophila* embryonic DNA by the chick sequence Cg45 and the two *Drosophila* sequences DCg1 and DCg2 are each composed of several cross-reacting species (Fig. 2). Although the three patterns are not identical, it is clear that they do overlap. The similarities are more apparent between Cg45 and DCg1 than between Cg45 and DCg2, supporting the idea that the *Drosophila* sequences may encode proteins of two different structural classes. The 1.2-kilobase (kb) internal *HindIII* fragment of pDCg2 (see Fig. 1) used as a probe in this experiment hybridizes to a single *EcoRI* genomic restriction fragment, but labels two *HindIII* genomic restriction fragments with approximately half the intensity of the *EcoRI* hybridization band. These data indicate that there are two closely related

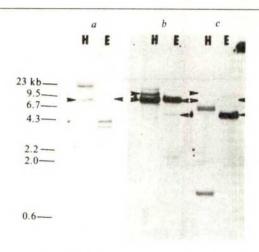


Fig. 2 Genomic hybridization patterns generated by Cg45, DCg1 and DCg2 sequences. Genomic DNA from *Drosophila* Canton S strain embryos (0–24 h) was digested to completion with either *HindIII* (H) or *EcoRI* (E), fractionated on 1% agarose gels and transferred to nitrocellulose filters²⁷. Each lane contained 5 μg DNA. ³²P-labelled probes were made from the cDNA insert of pCg45 by labelling with T4 DNA polymerase (Bethesda)²⁸ and from the DCg1 and DCg2 restriction fragments described in Fig. 1 by nick-translation²⁶. *a*, 1×10⁶ c.p.m. of Cg45 probe were hybridized at 37 °C (ref. 26) in the presence of 10% dextran sulphate²⁹ for 24 h. The filter was washed in 0.5 × SSC at 50 °C and autoradiographed for 4.5 days. *Drosophila* probes (2×10⁶ c.p.m.) from DCg1 (b) or DCg2 (c) were hybridized at 37 °C as in *a*, washed at 50 °C in 0.1×SSC, and autoradiographed for 2 days. The mobilities of *HindIII*-restricted λ phage DNA used as size markers are indicated. Some shared cross-reacting sequences are indicated by arrowheads.

sequences located on the same genomic EcoRI fragment, which may explain the size discrepancy between the EcoRI band present in both the phage isolate and our subclone (4.3 kb) and the EcoRI band labelled in the genomic hybridization experiment (5.6 kb). During cloning and propagation in the λ vector, a recombination event between the two regions of homology may have deleted a portion of the original genomic EcoRI fragment. Alternatively, an artificial EcoRI site may have been introduced into a larger genomic EcoRI fragment during the λ library construction.

The same Drosophila sequences that were used as probes in the above experiments were hybridized in situ to salivary gland polytene chromosomes. These experiments identified the cytogenetic locus of pDCg1 as 25C on chromosome 2L and the locus of pDCg2 as 19EF/20AB near the proximal end of the X chromosome (Fig. 3). Thus the two Drosophila sequences are located at separate, unique cytogenetic sites and are not part of one complex gene locus. The DCg2 sequence is located within or near a probable heterochromatin/euchromatin boundary. Other workers 17,18 have described active genes in analogous locations. The 25C locus for DCg1 is near a puff site in the 25AC region described by Ashburner¹⁹. This puff regresses in third instar larvae as ecdysone levels increase. This pattern of puffing is consistent with the developmental RNA profile of DCg1 described below. However, these similarities may be coincidental.

The accumulation of RNA transcripts homologous to the two *Drosophila* clones was measured in RNA populations representing sequential periods of the *Drosophila* life cycle (Fig. 4). A dot-blotting procedure was used in which aliquots of total RNA were immobilized on nitrocellulose filters and hybridized in probe excess (J.E.N. and B.J.M., unpublished observations). The maximal accumulation of homologous RNAs occurs during the first and second larval instars for both DCg1 and DCg2. RNA homologous to DCg1 is first detected during embryogenesis 12–15 h after oviposition, slightly preceding the first DCg2 hybridization signal. The presence of DCg2 transcripts extends beyond those of DCg1 into the third larval instar. RNA from cultured *Drosophila* Kc cells apparently contains transcripts

homologous to both DCg1 and DCg2. The hybridization and washing stringencies of these experiments demanded more precise base pairing than did the conditions used for the genomic blots in Fig. 2, and no significant cross-reaction occurred between DCg1 and DCg2 as measured by the DNA dots on each filter. The accumulation of DCg1 and DCg2 transcripts is clearly under developmental control.

Ultrastructural, biochemical 9.11,20 and genetic 21 studies in insects have demonstrated the existence of a basement membrane around internal organs in larval and adult stages, between the epithelium and muscle layer in larvae and also enveloping the imaginal disks. The developmental RNA profile that we have determined for the *Drosophila* sequences is consistent with the observed time courses for some of these processes, such as the initiation of internal or epithelial basement membrane formation during embryogenesis and early larval development. The pattern is equally consistent with larval cuticle deposition, although collagen-like proteins have not been reported to be components of this larval structure 22. These

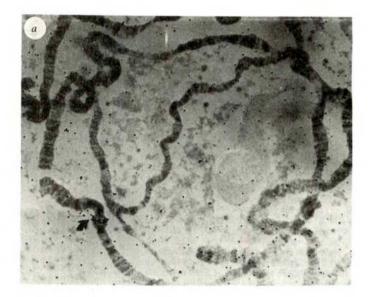




Fig. 3 In situ hybridization of DCg1 and DCg2 to Drosophila polytene chromosomes. ³H-cRNA probes were transcribed in vitro from DCg1 and DCg2 and hybridized at 60 °C to polytene chromosome squashes²⁶. Each slide received 4×10⁵ c.p.m.; 14-day exposures. a, DCg1 hybridization over 25°C on chromosome arm 2L; b, DCg2 hybridization over 19EF/20AB near the proximal heterochromatic region of the X chromosome. ×300.

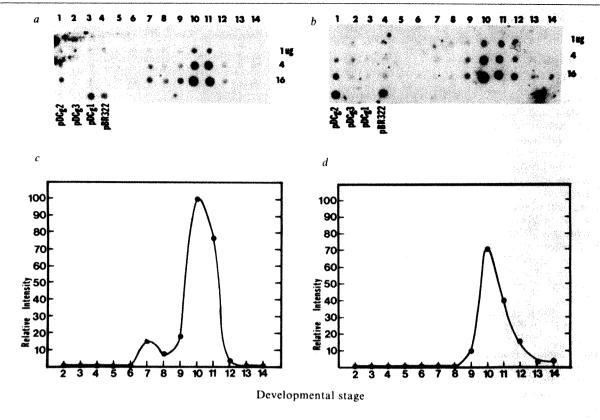


Fig. 4 Developmental mRNA profile of DCg1 and DCg2 expression. Total RNA was prepared from different *Drosophila* developmental stages³⁰ and immobilized on to nitrocellulose paper by loading aliquots in $20 \times SSC$ from a capillary pipette³¹. Three RNA concentrations (1, 4 and 16 μ g) were used for each developmental stage. Each filter also contained control dots of the plasmids containing the Drosophila collagen-like sequences. Filters were hybridized by applying sufficient probe sequences to generate a local 10-fold sequence excess over the highest estimated target sequence concentration in any dot (J.E.N. and B.J.M., unpublished results). Hybridizations were done at 42 °C in 50% formamide, $5 \times SSC$ hybridization solution 26 and the filters were washed in an equivalent solution at 50 °C after 24-30 h hybridization. The figure shows autoradiographs of RNA dot blots hybridized with DCg1 (a) or DCg2 (b) sequences. c, d, The autoradiographs were quantitated using an LKB soft laser scanning densitometer. The maximum absorbance was calibrated for the most intense hybridization signal of DCg1 and other signals are expressed relative to that signal. The developmental stages represented are: 1, Kc cells; 2-9, periods during embryogenesis following oviposition by 0-2, 2-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-21 h, respectively, 10-12, First, second and third instar larvae, respectively, 13, White prepupae. 14, Mid-pupation. An identical filter hybridized with pBR322 vector sequences showed hybridization only to control dots (data not shown).

sequences may also represent proteins containing a small, triplenelical region such as Clq23 or acetylcholinesterase24, although partial sequence data make this possibility unlikely for DCg1 ref. 5). Neither pDCg1 nor pDCg2 show evidence of in situ sybridization to the Drosophila acetylcholinesterase locus at 37E1-5 (ref. 25).

Characterization of collagen genes in invertebrates such as Drosophila will hopefully allow genetic analysis of collagen structure and expression as well as providing insights into evolutionary relationships of vertebrate collagens.

We thank H. Boedtker for providing the chicken cDNA clones pCg45 and pCg54; J. Fristrom and T. Kornberg for illowing us to use their fly facilities for collection of embryos, arvae and pupae; and T. Maniatis for providing the Drosophila ecombinant λ phage library used in screening. This work was supported by an NIH training grant to J.E.N. and an NIH grant o J.M.M. and B.J.M.

teceived 9 November 1981; accepted 2 February 1982.

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Errata

In the letter 'Chorus-related electrostatic bursts in the Earth's outer magnetosphere' by L. A. Reinleitner, D. A. Gurnett and D. L. Gallagher, Nature 295, 46-48 (1982), in Fig. 1 legend the words 'features of the chorus related electrostatic bursts. The slight inter-' have been repeated. Thus the second sentence should read 'The slight interference from 1738:45 to 1739:17 UT (also in Fig. 3) is from the ISEE electron density experiment.

In the article 'Protochordate allorecognition is controlled by a MHClike gene system' by V. L. Scofield, J. M. Schlumpberger, L. A. West and I. L. Weissman, Nature 295, 499-502 (1982), in Table 4 legend the percentage fusible progeny yielded by crosses where no alleles are shared should be 75%, not 7.5%

In the letter 'Stereochemistry of iron in deoxyhaemoglobin' by M. F. Perutz, S. Samar Hasnain, P. J. Duke, J. L. Sessler and J. E. Hahn, Nature 295, 535-538 (1982), in the final paragraph of page 537, references 14 and 12 should read 15 and 11, respectively. On page 538 the reference cited at the top of the second column of text should be 12, not 13.

MATTERS ARISING

The ν_3 fundamental band of the methyl radical

IN discussing near-IR absorption bands in IRS7, Allen and Wickramasinghe¹ remark that "laboratory data are not available for even quite simple, incompletely bonded molecules (for example, CH₃) such as might be found in the interstellar environment".

We have recently performed a laboratory measurement of the ν_3 fundamental band $(\nu_0 = 3,160.820 \text{ cm}^{-1})$ of CH₃ in absorption using a difference-frequency laser². The most prominent feature in the spectrum is the ${}^{\dagger}Q_0(N)$ (N=2,4,6,...)sub-branch. The transition frequency of strongest line, $^{r}Q_{0}(2),$ the $3,154.7459 \text{ cm}^{-1} \ (= 3.16983 \ \mu\text{m})$. Other strong lines at (300 K) are calculated to be: 3,101.045, 3,128.548, 3,199.730, 3,217.754, and 3,224.396 cm⁻¹. However, none of the prominent features reported by Allen and Wickramasinghe seem to fit our observed transition frequencies.

The methyl radical is expected to be an important species in certain interstellar sources³. In particular, estimates of the abundance of CH₃ in the atmosphere of the nearby carbon star IRC +10°216 indicate that its abundance relative to CO is $\sim 10^{-4}$ (ref. 4 and personal communications from E. M. McCabe, R. C. Smith and R. E. S. Clegg, and A. Kinney). Thus the methyl radical should be detectable in this source and we plan to conduct an IR search for it in the very near future.

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Saturn's rotation period

CARR ET AL. have inferred Saturn's rotation period from data recorded by the Voyager-1 Planetary Radio Astronomy (PRA) instrument. They constructed profiles of the received power in the coordinate frame of the Saturn longitude system (SLS) determined by ourselves? After comparing profiles made from month-long spans taken ~7 months apart, Carr et al. attributed a longitude shift to a 2-s error in the SLS rotation period.

Some problems in the work of Carr et al, affect the validity of their result. First is the precision of their method. From basic statistics we would expect the standard deviation in the individual rotation period determinations to scale with the square root of the number of samples. Thus, as Carr et al, used 2 months of data whereas we used 8 months, the former's individual standard deviation should be no better than root (8/2) times that of the latter, or ± 14 s. In addition, this value should vary with observing frequency owing to the change in emission occurrence probability with frequency. The rotation period at 500 kHz cannot be determined nearly as well as at 150 kHz because the occurrence probability is at least a factor of 4 lower at 500 kHz (ref. 3).

Using PRA data, we attempted to assess the inherent uncertainty in their method. We compared flux density longitude profiles generated over different time intervals, but at the same frequency. For example, comparing the longitude profile made at 346.8 kHz from data only 1 month apart, we found a 70° shift, which would correspond to an uncertainty of 15 s over the \sim 7 month time span used by Carr et al. We repeated the analysis at 155 kHz and at 97 kHz where we found that individual profiles were reliable to \pm 10 s and \pm 17 s, respectively. These are only estimates, of course, as we have not done an exhaustive analysis, but they are representative of the inherent uncertainty in determining the rotation period by aligning longitude profiles. Our error analysis² is sensitive to these large shifts, however, because it encompasses the entire span of data.

Second, we question the practice of combining results from several different frequencies to improve the precision of the determination. While this would be an acceptable statistical procedure in other circumstances, we have determined that the radio bursts are well correlated over a wide frequency range, and are never completely uncorrelated. Thus, receiver channels within the radio emission band contain non-independent, correlated information that cannot be averaged together, as was done by Carr et al., to reduce statistical fluctuations. We believe that conservative adherence to statistical principles would dictate that only one completely independent rotation period determination is possible using a single PRA data set.

Finally, and perhaps most importantly, the longitude profiles of Carr et al. were generated in a coordinate system locked to the Saturn-spacecraft line. However, because Voyager-1 moved 6° relative to the Saturn-Sun line during the 7-month analysis interval in question, the data should instead be organized in fixed (sub-

solar) coordinates. This angular shift will manifest itself in the longitude profiles, and hence in the rotation period, and should be taken into account. In short, given the inherent imprecision of the histogram method, the Carr et al. determination is certainly not statistically inconsistent with ours.

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CARR ET AL. REPLY—The strongest objection of Kaiser and Desch to our results is essentially that we used histograms plotted as a function of the saturnian central meridian longitude of the spacecraft instead of that of the Sun in our cross-correlations. When our oversight is corrected, we find our three rotation period measurements to be only 1 s less than they were before, and their average to be 3 s instead of 2 s less than the SLS value. This small correction does not change the situation materially.

We agree that the SLS rotation period measurement is probably more accurate than ours, but we do not believe that the difference in accuracy is large. The agreement to within 3 s increases confidence in both determinations. Our accuracy can certainly be improved by a better choice of data interval for the first histogram in each pair. We do not agree with the contention of Kaiser and Desch that little is to be gained by using data from several channels instead of just one. The redundancy resulting from the high correlation between pairs of closely spaced channels indeed tends to reduce their individual contributions to the overall precision, but is beneficial in other ways such as minimizing the effects of data gaps and of interference incorrectly identified as Saturn. The best method, in our opinion, would be to calculate the rotation period using data from each of about 10 selected channels separately by the method of Desch and Kaiser, and then to combine these values, appropriately weighted, into a single average.

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BOOK REVIEWS

Prehistoric geometers?

J.N. Graham Ritchie

STONE circles and standing stones have perhaps a greater fascination than most other classes of prehistoric monument, not because we understand more about them, but because we know less. With hill-forts, burial mounds and hut-circles we can readily appreciate their possible role within society; with stone circles and standing stones this is not so, and excavation does not add greatly to an understanding of their function. The archaeologist may discover burials at the centre of a circle or cremated bone within the packing of the socket of a standing stone, but he cannot say that burial was the only function of such sites, and the suggestion that circles may have been central places for the dissemination of legal or religious lore is a woolly alternative.

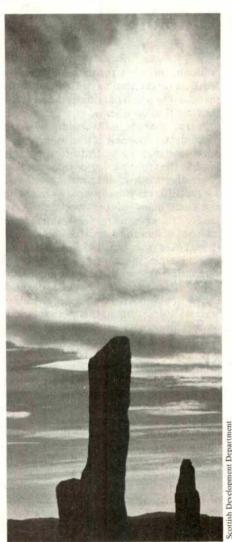
The most comprehensive interpretation of many megalithic remains has been put forward by Alexander Thom in a series of papers and books resulting from years of painstaking fieldwork and observation, particularly in Scotland, Wessex and Brittany. Thom has suggested the use of a standardized unit of length in their construction and a knowledge of complex geometry in their layout. Further, he has postulated that the erection and positioning of stones and cairns was designed to allow sophisticated observation of the major celestial bodies, including the prediction of eclipses. Thom's work, together with the influence of Gerald Hawkins and J.B. White's volume Stonehenge Decoded (Souvenir, 1965) in which the famous circle was described as an observatory, have created a climate of popular opinion in which the concept of prehistoric scientists and astronomers is accepted without question. How can you prove, the archaeologist is asked, that such people did not exist, or are less credible than purveyors of social or legal wisdom - classes in prehistoric society that seem to be taken for granted.

Perhaps it takes a scientist to examine a potential prehistoric colleague; in this masterly volume, Douglas Heggie, a mathematician and astronomer in the Department of Mathematics at the University of Edinburgh, has explained clearly the data on which prehistoric science rests. It is as though Heggie, the teacher, has returned to prehistoric times to test the knowledge of a student of science and astronomy. The possible range of the background information that the student might have is carefully set out in non-scientific language; whether the

Megalithic Science: Ancient Mathematics and Astronomy in North-West Europe. By Douglas C. Heggie. Pp.256. ISBN 0-500-05036-8. (Thames and Hudson: 1981.) £12, \$27.50.

student possesses this knowledge is then judiciously weighed and statistically tested, but the conclusion — to the sadness I suspect of the examiner and the reader — is that he did not have many of the skills that had been attributed to him.

Heggie has set out the evidence for the units of length which may have been used in the construction of stone circles, and has explored the ways in which the sometimes deliberately eccentric ground-plans of such sites were designed. Using a rigorously



Clouded past — part of the stone circle at Callanish, Lewis, in the Western Isles.

statistical approach Heggie finds little evidence for a highly accurate unit of length (his italics), and while accepting that Thom's classification for the shapes of megalithic sites is helpful, he questions the need for any knowledge of Pythagorean triangles and of π in their layout. Heggie's conclusions on astronomical matters are equally tentative: that, while prehistoric man knew something of the movements of the Sun, and possibly the Moon, his role was that of an observer rather than theoretician.

Such a verdict does not lessen the achievements of early man as an architect and engineer, and perhaps increases the importance of those particular aspects of his astronomical knowledge that are found to exist. Indeed our appreciation of his strengths in empirical science are increased by such careful examination; perhaps most remarkable is the orientation of the passage grave of Newgrange, County Meath, so that the rising Sun of the winter solstice bathes the rear chamber with light, not through the entrance to the tomb, but through a specially designed opening, known as the "roof-box". Thus respect for the many empirical skills demonstrated by the designers and builders of stone circles and alignments is not called into question by doubts about their appreciation of theoretical science or involvement in astronomical observation to a high degree of complexity.

A subject rarely discussed in archaeoastronomical literature, and not considered in detail by Heggie, is that of the interpretation of the archaeological evidence itself. Distinct classes of stone monument can be outlined, some with a long chronological span, others covering shorter periods, and still other groups, such as simple upright stones, the dates of which remain quite unknown. Archaeologically it is important to distinguish between those sites where the upright stones delimit an open area, such as most stone circles, and those where the stones have acted as the retaining kerb of a burial cairn. The complexities of megalithic sites can be demonstrated most fully in terms of structural alteration over perhaps two millennia by the recently excavated site of Temple Wood in Argyll and our knowledge of this sequence is now much greater than that outlined in this volume. Where astronomical observations have been to the highest standard (in Thom's work this is not in question), it is unfortunate that the archaeological interpretation of archaeo-

astronomers has not been as scrupulous indeed some stone alignments given astronomical interpretations may be no more than the foundation stones of turf field-walls where the ephemeral materials have weathered away. The lack of scientific knowledge for which field archaeologists are so often criticized has led to a disregard for those skills of observation and site interpretation that are their own stock in trade. A reassessment of the archaeological content of Thom's work - for in the final analysis it is on the stones themselves that the hypotheses rest — would have been a valuable adjunct to Heggie's reexamination of the data.

It is important to stress that this discussion is concerned geographically with north-west Europe and chronologically with the period between about 4000 BC and 1000 BC. Considerable momentum for the study of ancient astronomy in other parts of the world has resulted from Thom's work, though not directly stemming from it, and the levels of observational astronomy practised in other societies will clearly have a bearing on what we expect

from our present vision of prehistoric Britain. Nor will all scholars view the evidence in the same way — there is no simple "standard archaeological model", no "orthodox archaeological picture" to compare with a map of the stars; most models change with new discoveries and new approaches, either imperceptibly or radically.

Few debates can have been as measured and carefully presented. Professor Thom and his son Dr A.S. Thom have outlined in clear steps and precise documentation the basis of their findings on megalithic measurements, shape, astronomy and calendrical matters. Dr Heggie has used this material and considered it in mathematical, astronomical and statistical ways, and has presented his own findings with caution and fairness. His ability to disagree without minimizing the work of others is perhaps his greatest personal contribution.

J.N. Graham Ritchie is an Archaeological Investigator with the Royal Commission on the Ancient and Historical Monuments of Scotland.

Virus variety for plant pathologists

Milton Zaitlin

Handbook of Plant Virus Infections: Comparative Diagnosis. Edited by Edouard Kurstak. Pp.943. ISBN 0-444-80309-2. (Elsevier/North-Holland Biomedical: 1981.) Dfl.395, \$192.75.

A GIVEN virus often causes disease in several different plant species. Thus although well over a thousand plant virus-induced diseases are known, they are caused by only several hundred viruses (350 are considered in this handbook). This situation is further clouded by the assignment of unique names to identical diseases discovered independently.

A good conceptual framework for virus classification is therefore essential. Fortunately, ten years ago a rational approach to the problem was put forward by a small group of plant virologists in which viruses were collated into 16 "affinity" groups based on their morphology, particle composition, mode of transmission and so on. Quasi-official sanction has been given to this movement by the International Committee on the Taxonomy of Viruses, which has undertaken responsibility for the classification of viruses in general. The classification is periodically reviewed, modified and extended, and every three years, after the meeting of the ICTV, the revised classification is published as a small volume.

Edouard Kurstak's handbook follows the ICTV classification scheme, providing detailed individual chapters on virus groups, each prepared by a specialist. It is an extremely useful reference work. In addition, there is a perceptive chapter by R.I.B. Francki dealing with plant virus taxonomy in which he outlines the basis for the classification scheme.

A true "handbook" should provide as much data as possible if it is to serve as a good reference work. Unfortunately this book suffers to some degree from uneveness in the depth of information covered for the different virus groups. As might be expected, many of the specialists have tended to emphasize their immediate interests within their assigned group, and they cover the available information beyond their specialities with differing emphasis. As one extreme example, six pages of text are devoted to a detailed listing of the hosts of the hordeiviruses, whereas little information is given for some other groups with extensive host ranges (such as the potyviruses) where such information is available. The editor is not himself a plant virologist and it is understandable that he would have to rely heavily on the judgements of the individual authors as to which topics should be emphasized.

All in all, the book is excellent and would be a welcome addition to the bookshelves of agriculturists, and to plant pathologists in particular. It is especially well-produced on quality paper and contains a great deal of tabulated information and many good photographs. Certainly, I will treasure my complimentary copy.

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Something in the air

J.E. Lovelock

Atmospheric Sulfur Deposition: Environmental Impact and Health Effects. Edited by David S. Shriner, Chester R. Richmond and Steve E. Lindberg. Pp.568. ISBN 0-250-40380-3. (Ann Arbor Science Publishers/Butterworth: 1981.) \$29.50, £17.

As in the workings of a Greek tragedy, there is nothing now to indicate the discomforts that lie ahead as heedlessly we burn fossil fuel and CO2 grows ever more abundant in the air. But this is not so for the small percentage of sulphur in these fuels; of all consequences of mankind's industrial activity on the planetary environment, none are more obvious than those caused by the emission of sulphur compounds to the atmosphere. Their presence is objectionable on scales running from the stench of reduced sulphur compounds locally released, to the planetaryscale effects on climate of the Junge stratospheric sulphate aerosol. In between these lie other consequences, such as the breath-taking lethal smogs which once beset London and the translucent sulphate aerosol which obstructs the summer sunshine over whole regions. Worst perhaps is the destruction wrought by acid deposition which diminishes life in the forests, lakes and rivers of much of the Northern Hemisphere.

Atmospheric Sulfur Deposition is an unusually tight and well-edited collection of papers from a symposium held at the Oak Ridge National Laboratory in Tennessee. It moves from the consideration of the costs and benefits of controls and their legislative implementation to the little that is known about natural sources of sulphur gases in North America, and then to the emissions from the technosphere. The atmospheric chemistry, the transfer across the interfaces with the land, and ocean, the effects good and bad on man-made and natural ecosystems, all are taken into account.

It is a book for those sizeable ranks of professionals concerned with national and regional air pollution problems. These include scientists and administrators in government and industrial service, and also legislators and regulators. For this group it will provide a very credible addition to their working knowledge of the sulphur pollution problem. The book is not primarily intended for those scientists curious about the great natural cycles of the elements and who might wish to know more about the part played by sulphur. Even so, this group also might well benefit

Missing from the review of recent textbooks of invertebrate zoology (Nature 295, 482; 1982) was mention of Peter Calow's Invertebrate Biology: A Functional Approach. Published by Croom Helm, the book costs £11.95 (hbk), £5.95 (pbk).

from reading it, for it establishes the context in which so many papers on the atmosphere and on atmospheric pollution are written, a context very different from the rarefied altitudes of academic aeronomy.

The natural cycles of the elements are closely linked and it is usually difficult to consider one of them in isolation. This restriction limits the value of the papers on the natural sources, but in any case information about natural sulphur emissions is still very meagre. The presence in the environment and the release of such compounds as COS, CS₂ and alkyl sulphides has only very recently been established. To be certain of the extent of their contribution to the natural cycle of sulphur we must wait until expeditions have measured the abundance and release rates of these transient and important compounds. It is not enough to rely on the few measurements made at sites close to the northern industrial regions. To be representative of the sulphur cycle in space and time we need measurements from all parts of the planet. made so as to include the diurnal, seasonal and secular variations. It is also possible that other sulphur compounds are yet to be discovered. Indeed, the presence of methanesulphonic acid in the sulphate aerosol has been reported. This compound, an end-product of the atmospheric oxidation of biogenic sulphur gases, may be a useful indicator of the proportion of acid deposition from these sources as compared with that coming from combustion.

There is a tendency in the book to assume that the agricultural ecosystems which cover most of the land surface are "natural" and that emissions of sulphur gases now measured from the soil are the same as those from the pre-industrial and pre-agricultural ecosystems. It now seems at least possible that the contemporary releases of sulphur gases are the product of a pathology and a consequence of a surfeit of sulphur and of other nutrients. This comment applies also to the rivers, estuaries and salt marshes which are the recipients of the run-off from agricultural and urban sewage.

Truth is said to be the first casualty of war. Looking back on the parallel environmental issue of the ozone war it seems also that scientific objectivity does not flourish in the sparring which precedes legislation and regulation. Inevitably, any collection of scientific papers from a meeting about an atmospheric topic whose objectives are national rather than global will reveal some limitation of this kind. This book commendably has overcome such a tendency to parochialism; thus the European dimensions of the acid deposition problem are well represented, and an attempt is made to link with more general scientific aspects through papers on the natural sources of sulphur gases.

Antibiotics at work

H.R. Perkins

The Molecular Basis of Antibiotic Action, 2nd Edn. By E.F. Gale et al. Pp.646. ISBN 0-471-27915-3. (Wiley: 1981.) £35, \$78.

SECOND editions can represent a special problem for reviewers, particularly if the movement in the subject matter (or worse. in the presentation of it) has been slight. Let me say at once that any difficulties in reviewing this particular production have arisen from precisely the opposite cause, namely that the nine years separating it from its predecessor have seen great advances in ideas about how antibiotics work. Even by the exercise of what must have been miracles of compression, the authors, all members of what one might term the Cambridge School of antibiotic investigators, have only succeeded in containing their subject within some 50 per cent more pages than before. That they have done so is a great credit to them; the book remains readable and transportable. essential characteristics of any text that has not become enshrined as an encyclopaedia.

The form of presentation remains unchanged. A general introduction to the basic concepts of antimicrobial substances is followed by logical and extensive chapters centred around the major targets of action, namely microbial wall synthesis, membrane function, nucleic acid synthesis and ribosome function. A consideration of bacterial resistance to antibiotics follows and the epilogue is entitled "Perspectives". The unifying approach is very valuable to the reader, who might be anyone from an honours microbiology student with an exceptional (or heavily directed) thirst for knowledge to a research worker seeking a detailed introduction to the field. Each chapter is well illustrated with tables and figures, and has a bibliography so generous and so recent that only an aggrieved author failing to find his own gems therein could possibly complain.

Of the three volumes covering the same general field of interest that I have reviewed recently, this is the most "up-market" in intention and there is no doubt that it achieves its object admirably. It will appeal particularly to professional chemists, biochemists and microbiologists who require a clear presentation of the latest word on the interaction of antibiotics with their targets. The coverage of information that has accrued since the first edition is impressive. When one considers that, for instance, transposons were unheard-of then and penicillin-binding proteins had not been isolated or characterized, one realizes the extent of progress that has been made in our understanding of the molecular mechanisms underlying antibiotic action and microbial resistance. Whoever purchases the present volume has an exceptional opportunity to rectify any deficiencies in his fund of information.

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Only if he wishes to know about developments in the antiviral field is he likely to go away more or less empty handed, but since the authors have largely, though not by any means entirely, confined themselves to "antibiotics" in the strict sense, this particular deficiency may be easily forgiven.

H.R. Perkins is Professor of Microbiology at the University of Liverpool.

Truncation at length

Francisco J. Ayala

Genetic Variability. By Christopher Wills. Pp.312. ISBN 0-19-857570-X. (Clarendon/Oxford University Press: 1981.) £24, \$49.50.

THE existence of inheritable variation is, as Darwin saw it, a necessary condition for evolution. The genetic variants present in a population at a given time define the evolutionary changes that are possible in the population. It has become established during the past two decades that populations of all sorts of organisms store an enormous wealth of genetic variation. In this book, Christopher Wills, a distinguished population geneticist of the University of California at San Diego, deals only in passing with the issue of how much variation is there, but explores in depth the mechanisms that account for the variation

This is a roman à clef. Wills's argument is that genetic variation is maintained largely by natural selection operating according to the truncation model. We may envisage the distribution of organisms according to their phenotypic fitness. A certain proportion of the organisms will survive and reproduce. According to the truncation model, the fitness value below which no survival occurs is not absolute but depends on the actual fitness distribution. The implications of truncation with respect to many central problems of population genetics are investigated at length, and most are published here for the first time this is undoubtedly the most significant contribution of the book. Among the important conclusions reached, one is that the gene is the unit of selection (Chapters 8 and 9). With truncation, the strength of linkage disequilibrium in outbred populations is not a function of selection but of the population size.

According to Wills, the most compelling evidence in favour of truncation selection

François Jacob's The Logic of Life: A History of Heredity (reviewed as The Logic of Living Systems in Nature 251, 81; 1974) has been reissued by Pantheon Books, New York. The book includes a new preface by the author, and costs \$7.95 in paperback.

is negative. If the fitness effects of each locus interact multiplicatively, then the maintenance of multiple polymorphisms would require a considerable segregational load — "highly homozygous populations should be extremely unfit' Astonishingly, on the following page Wills states that this is "contrary to observation". This ignores a number of experiments (e.g. M.L. Tracey and F.J. Ayala, Genetics 77, 569-589; 1974) showing that, when fitness as a whole is measured under population conditions, the inbreeding depression is enormous sufficiently large to account for hundreds of polymorphisms even if fitness interactions are multiplicative.

Wills asserts that whether or not most genetic variation is selectively neutral is a question "impossible to resolve, since it is impossible to prove the absence of something" (p. vii). This last phrase is obviously wrong, since I can prove to the

satisfaction of any reasonable person that there is no 747 Boeing airplane in my office. Most importantly, the statement misconstrues the aims and methods of science. The neutrality theory has been extremely fertile because it makes certain predictions about genetic variation, rates of evolution and so on. If such predictions were correct, the theory would be corroborated. Natural selection might be involved, but if it could be ignored in accounting for the relevant phenomena. there would be no reason to incorporate it in our explanations. One need not take into account the craters of Venus when explaining planetary motions. The point is a substantive one: the only hypotheses and the only parameters that are acceptable in science are those that are necessary in order to account for empirical phenomena.

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A time and place for fire and brimstone

Peter J. Smith

Volcanoes of the World: A Regional Directory, Gazetteer, and Chronology of Volcanism during the Last 10,000 Years. By T. Simkin et al. Pp.248. ISBN 0-12-787478-X. (Hutchinson Ross: 1981.) \$19.75, £13.20.

PEOPLE have been compiling lists of volcanoes since at least 1650, when Varenius tabulated 27 examples. Two centuries later, Humboldt raised the total to 407; and, when complete, the IAVCEI's Catalog of Active Volcanoes (begun 1951) will contain details of about 900. The magnificent Smithsonian Institution volume under review here, however, lists no fewer than 1,343 volcanoes known or likely to have been active during the Holocene and 5,564 dated eruptions from about 8300 BC onwards, all arranged regionally and chronologically, respectively, in two large tables, to which are added a gazetteer and a bibliography.

Volcanoes of the World is thus the most complete and easily accessible compilation of volcanoes and volcanic activity now available, although the authors themselves hardly dare breathe the word "complete" in this context because their listings are anything but. For a start, most volcanism takes place on the sea floor and almost all of that goes unnoticed. Even on the continents, however, there are still regions in which much volcanic activity goes unrecorded; and if that can happen in a highly populated world shrunk by modern communications, how much more extensive must have been the omissions of the past. Indeed, Simkin and his colleagues demonstrate graphically that both the total number of known volcanoes and the

number of known active volcanoes have increased roughly exponentially over the past 600 years, which suggests either that the Earth is about to be overwhelmed by volcanic activity or that the perceived volcanic state of the planet is highly dependent upon reporting efficiency.

Under such circumstances, attempts to correlate known historic volcanism with other phenomena must be undertaken warily. Even so, the compilations at the heart of this volume will be a boon to a remarkably wide range of scholars quite apart from geologists. Biologists interested in colonization will find here the 96 eruptions known to have formed islands; glaciologists can easily pick out the 15 volcanoes with known subglacial eruptions; agriculturalists may like to know of the 181 volcanoes with eruptions known to have destroyed arable land; climatologists will look for those volcanoes known to have ejected particularly large amounts of ash and dust into the atmosphere; anthropologists might examine the listed eruptions known to have severely affected communities; and so on.

In short, Volcanoes of the World is, a splendid work of considerable interdisciplinary value and will become even more so if, as the authors hope, feedback from readers and other interested parties enables some of the many gaps to be filled. For this is not a one-off job but the first step in a continuing attempt to build as extensive a file as possible on the statistical aspects of the world's volcanism.

Peter J. Smith is Reader in the Department of Earth Sciences at the Open University, Milton Keynes, and editor of Open Earth.

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nature

1 April 1982

Mycotoxins in South-East Asia?

The US government says that chemical weapons have been used in Indo-China, but its evidence falls short of proof. Even so, there is a case to be answered. How?

Last week's account by the State Department of chemical warfare in South-East Asia is a little like the discovery of quasars: something is undoubtedly going on, but it is impossible to tell just what. Part of the difficulty is that there is so little objective evidence. The principal allegation (see Nature, last week) is that the Vietnamese have been using chemical weapons of some kind in the wars in which they have been or are still engaged in Laos and Kampuchea (Cambodia), that the chemicals concerned include toxins of the kind produced during the growth of fungi of various Fusarium species and that these have been manufactured in the Soviet Union. The allegation, already vigorously denounced as "propaganda" by the Soviet news agency TASS, was first made public by Mr Alexander Haig in Berlin last September, but was at the time almost entirely unsubstantiated. Since then, scraps of information have been squeezed like blood from a stone from the State Department. Last October, the State Department gave the ad hoc group of experts set up to investigate the charges by the United Nations the results of the chemical analysis of samples of vegetation and other materials said to have been collected from Laos and Kampuchea. Now, it seems, there is more to say. The State Department clearly thinks it has evidence with which to prove its case. But only four samples of supposedly contaminated material have so far been analysed in the laboratory. The circumstances are exactly analogous to those in the early days of the quasars, when the implications of a meagre stock of data were clearly so huge that people could not summon up the fortitude to follow the prudent course — to wait for more data to accumulate. What, in these circumstances, is to be made of the State Department's report?

Although there are some numbers to play with, anecdotal evidence remains the most compelling part of the State Department's case. Reports of interviews with refugees from Laos and Kampuchea now in camps in Thailand have several strikingly common themes. Over a period of five years, they say, villages in central Laos and on the Kampuchean border with Thailand have been attacked with sprays, bombs or rockets releasing toxic agents. The heads of the rockets are said to have released red, blue or white smoke, while the sprays are now widely known as the causes of "vellow rain". The reports of the damage done to people are too many to be overlooked — or to have been fabricated. Nausea, dizziness and respiratory and circulatory troubles are the milder symptoms. Bleeding gums and other haemorrhagic phenomena are common. Statements that the corpses of those who have died are often bloated, and that their digestive tracts have rapidly become bloated, are a common theme in the reports of these first-hand accounts of attack from the air. And the State Department's star witness, a Vietnamese pilot who defected in 1979, has given such a specific account of how he carried out air attacks with unusual and presumably toxic

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weapons that those wishing to declare it false could fairly be asked to provide a specific and not a general denial.

Like all anecdotal evidence, of course, accounts such as these, however gripping, do not constitute proof. Interviews conducted by intelligence officers in far-away refugee camps do not have the objectivity even of the kinds of conclusions that emerge from examinations carried out by lawyers in courts of law. Indeed, in South-East Asia, the process of gathering verbal evidence must have been made even less certain by the difficulty encountered by the United Nations expert group on its flying visit to Thailand at the end of last year — the need to translate from a local dialect into Thai and from that into English. From the evidence now published, there is no way of telling how far witnesses may have been led to a desired tale by the questions asked of them, no simple way of making sense of statements that do not ring true (such as the assertion that yellow rain was sprayed from a height of 7,000 feet) and no simple way of being sure that the time-course of the conditions caused by the supposedly toxic chemicals were accurately described. The weight of the verbal evidence that toxic weapons have been used in South-East Asia therefore rests on the amount of it, not on its quality.

Because of such doubts, objective scientific evidence is potentially crucial. Here the difficulty is that there is far too little of it. Only four samples of material have so far been analysed for trichothecenes, the toxins produced by Fusarium species. According to the laboratory of Dr Chester J. Mirocha of the University of Minnesota, where the analyses have been carried out, the figures given in the State Department's report represent the proportions of trichothecenes in the whole bulk of the samples which have been analysed, so that if a leaf or water has been contaminated by some artificial external agency, the concentration of the toxins in that source must be higher, probably very much higher, than the concentrations that have been reported. In any case, the nub of that part of the State Department's case that rests on the data now made public, is that the concentrations now reported are greater than those found in grain that has been contaminated with Fusarium fungi in natural circumstances. The comparison seems to be generally accepted. Even allowing for the improved methods of analysis now available, it is unlikely that the samples recovered from South-East Asia have been contaminated by the natural growth of Fusarium species on the spot. But even this is not the clinching argument the United States State Department would like it to be.

The several objections to the use of these data will seem pettifogging to those who know what case they want to prove, but are nonetheless worth stating openly. First, four samples of such small bulk and of such different kinds are hardly enough to demonstrate the internal reproducibility of the results now arrived at. Worse still, those responsible for the chemical analysis have no knowledge of how the samples they have dealt with were collected, stored and transported. Indeed, they can have no way of being sure that the mycotoxins whose presence they have demonstrated were not added to the samples by some agency or individual anxious that the State Department should have a persuasive tale to tell. This dark suspicion, which nay seem unjust to a responsible government such as that of the United States, cannot unfortunately be shaken off, for the intelligence community responsible for collecting the samples has a shaky

reputation for veracity. The only solution to this dilemma is, of course, that those responsible should acknowledge that this potentially objective part of their case will be convincing only when there is much more material for analysis whose authenticity can be demonstrated.

For the time being, the analytical evidence falls far short of being compelling, at least within the professional community. The point can be simply put by means of the conventions of the scientific literature. Thus the State Department has agreed that Dr Mirocha and his colleagues may, if they wish, publish the results of their analyses in the scientific literature. (So, of course, they should do.) The abstract of an acceptable contribution to the literature along these lines might take the form:

We have analysed four samples of materials supplied by the State Department and have obtained the following results. . .

Referees would no doubt say that such a report was technically beyond reproach but of somewhat limited interest. But if the proposed publication has an abstract beginning:

We have analysed samples collected from four sites in South-East Asia soon after the use of chemical weapons... and have concluded that the Vietnamese have been using toxic agents derived from Fusarium species....

the referees would be down on them like a ton of bricks. This does not imply that the State Department's case falls down but rather that even with the apparently objective evidence for it now adduced, the case remains largely circumstantial.

The State Department has not yet proved its case, but it has raised a question that cannot be ignored. It is in everybody's interest that it should be answered, and quickly. One of the most chilling passages in the State Department's report is the speculation — it is no more — that mycotoxins rather than more lethal agents have been used in South-East Asia because they lead to bizarre forms of death calculated to frighten away the survivors of attacks. (Certainly the refugee camps of Thailand have been full for months.) If indeed it has now been shown that chemical weapons like these do have some military value, the outlook is awesome not merely for the luckless people of Laos and Kampuchea but for the rest of us. The prospect that it might be possible to reach an agreement between the major military powers to outlaw the use of chemical weapons in future wars, and to abandon their manufacture, will clearly be diminished if some group of generals has it firmly in its collective head that such a treaty would be a military loss.

So what should be done? The United Nations group of experts should clearly be asked — and helped — to complete the study on which it has embarked. The practical need is that it should be able to make a more detailed investigation of the regions in South-East Asia in which mycotoxins are said to have been used. The Soviet Union, which has some influence with the government of Vietnam, should recognize that its own case — that the allegations are a pack of lies — could only be strengthened by an investigation on the spot. And the United States should understand that the glee with which it has accused the Soviets of complicity in whatever has been happening in South-East Asia is counterproductive. Thus it serves no purpose but to confuse, that the State Department's report says that techniques for manufacturing and spraying trichothecenes from the air have been developed in the Soviet Union without adding that the objective (fully described in the literature) has been to control the spread of fungi other than Fusarium and related genera in forests. One of the materials, the trichothecene T-2, has even been canvassed as a rodenticide. (Nobody suggests that it would make sense to think of killing rats by spraying such a material from the air.)

What the State Department has not appreciated is that the question which it has legitimately asked will be regarded less seriously by those who alone can provide an objective answer if the statement of the problem is biased. The danger that the appearance of the State Department's document will be mistaken for a political ploy is in any case enhanced because the United States government is in the thick of persuading Congress to embark on the manufacture of binary chemical weapons in the

form of the 155 mm artillery shell known as M687, rightly castigated last week by Dr Matthew Meselson of Harvard University (before a Senate subcommittee) as militarily pointless and politically inflammatory. At the same time, to be fair, President Reagan has asked that the United States delegation to the Committee on Disarmament in Geneva reopen discussions on measures to control chemical weapons. The zeal with which those talks are prosecuted will be a powerful determinant of people's willingness to answer the State Department's question.

Professional propaganda

By what means should professional people, not only scientists, aim to influence public opinion?

The professions have often been accused of indifference to the problems of the societies in which they are embodied, and there is substance in the charge. Now, when many industrialized states are faced with unfamiliar and serious economic crises, governments may often rightly grumble that academics are more concerned with their own skins than with the solution of the economic problems about them. Other professions are similarly indictable. During the housing boom of the 1960s, for example, architects built dwellings without recognizing that people might not want to live in them, while teachers have for decades taught in schools without paying sufficient attention to the fitness of the curriculum for their students' needs. Not all, however, is disappointment. In many places, the legal professions have taken the lead in linking the practice of the law more closely with social realities and needs, while physicians have done much to change people's attitudes towards health and disease. So is it not entirely to be welcomed that there has recently been a rash of professional groups taking a special interest in what may be the most serious of

all contemporary social problems — the dangers of nuclear war? The short answer, which is "no", appears to offend some of those concerned (see page 386). That professional people have a responsibility to contribute their special knowledge to the solution of general problems is not in dispute, but only the means by which they do so. Similarly, there is no question that when a profession is, broadly speaking, agreed about the social consequences of some circumstance lying largely within its field, it should declare itself. It would thus be shocking if physicians were silent about the probable consequences of smoking. Unfortunately, however, the dangers of nuclear war do not lie exclusively within the province of a single profession, while there can be very few professions that are in broad agreement within themselves about the steps be taken to head off the danger.

This is why the emergence of groups of architects, or teachers, or even scientists, with catchy acronymic titles "against nuclear war", is a misfortune. By suggesting that the whole of a profession is in some way united about what should be done, these groups give the general public a false impression. By being separate from the more broadly based groups of people with the same aims, they run the risk of over-simplifying important problems. Medical groups, for example, may fairly make fun of official plans for civil defence against nuclear attack, but it would be more constructive if they did so in the context of the appalling difficulties, political and otherwise, of avoiding dependence on nuclear weapons.

In Britain and elsewhere in Western Europe, the immediate question is not whether nuclear war would be devastating—everybody is agreed on that—but whether individual governments should to some degree opt out of present arrangements for the defence of Europe. Another question is the basis on which arms control agreements might be negotiated. Professional groups usually profess no opinion on such practical questions, preferring to stay within the confines of their professional competence. The result is that, by what they say, they influence general opinion irrationally or at least subliminally. This is why they are open to the charge of deceit.

Industry funds in universities

New guidelines emerge from Pajaro Dunes

Washington

Universities should only accept research funds from private companies if secrecy is kept to the absolute minimum necessary for patent protection. At the same time, companies which fund such research should normally be entitled to receive exclusive licences on any useful results that emerge, at least for a period sufficient to prevent rival companies from unfairly exploiting the same research.

These were two of the principal conclusions to emerge from a three-day meeting between the presidents and selected faculty members of five top US research universities, and senior executive officers of ten leading biotechnology companies, held at the California coastal resort Pajaro Dunes at the end of last week.

The conference had been organized largely at the suggestion of Dr Donald Kennedy, president of Stanford University. Its main purpose was to discuss a range of controversial issues that have emerged over the past few years as universities have looked to industry as an alternative source of research support from the federal government and have increased their efforts to push research results into the market-place. Companies in turn have been turning their attention to the frontiers of biomedical research in their quest for new and improved products and industrial processes.

Others attending the conference included the presidents of Harvard, Massachusetts Institute of Technology, University of California and California Institute of Technology. Each had been asked to invite one university administrator, two faculty members with direct or indirect experience of dealing with outside companies, and two senior executives from companies with experience of sponsoring university research. The companies represented at the meeting ncluded Beckman Instruments, Syntex, Cetus Corporation, Applied Biosystems nc., Gillette Corporation, Eli Lilly, DuPont and Genentech.

Dr Kennedy stressed after the meeting hat the purpose had not been to agree on igid rules that should apply to each inversity, but rather to work out what he lescribed as a "framework for future elationships between universities and industry". Faculty members at several of he universities represented at Pajaro Dunes had already indicated their concern hat it should be left to each university to

decide how broad principles should be interpreted into policy — a policy that last week's meeting was quick to endorse.

Nevertheless, criticism that the conference had been limited to senior administrators and scientists on both sides was expressed in a letter to the participants signed by 25 scientists at research universities across the country, as well as several prominent union and consumer spokesmen such as Anthony Mazzochi of the Oil Chemical and Atomic Workers, and Ralph Nader.

The letter invited the participants to attend a second conference later this summer, at which the same topics will be discussed but primarily from the point of view of groups both within and outside universities which felt they had been unfairly excluded from last week's meeting. The letter quoted from the final speech delivered by President Eisenhower, in which he criticized not only the growing power of the "military-industrial complex" but also the danger that important policy issues were increasingly

being decided by a scientific and technical elite, rather than through open democratic processes.

Dr Kennedy has already agreed to take part in this second conference. He also said on Saturday that he would help the organizers of the conference to raise the necessary funds.

The Pajaro Dunes meeting produced agreement on an 11-page statement which set out some principles as a basis from which individual universities can develop guidelines and codes of conduct. The statement stressed, for example, that although links should be encouraged between faculty members and outside companies since these were considered mutually beneficial, "professional relationships with commercial firms should not be allowed to interfere with responsibilities for teaching and research"

The statement also said that in general it was not appropriate for universities to own substantial equity in companies which were staffed by their own faculty members. This is a sensitive point at Harvard, which

Flying start towards French law

The proposed French "research law", on which many of the plans of the minister for research and technology, Jean-Pierre Chevènement, depend, has cleared its first hurdle with a flying leap. The Economic and Social Council — a kind of litmus paper of the French nation — was not satisfied with giving mere approval to the law last week. Rather, it rearranged and added sentences to the draft bill to stress the significance of the new plans for France, indicating that they must be considered to be political priorities.

This is no slight thing, as the finance minister, Jacques Delors, recently put all spending ministries (including Chevènement's) on a tight rein. Something like a quarter of the new money offered to laboratories by Chevènement has had to be frozen, despite the minister's strong opposition. But, says the Economic and Social Council, "expenditure on research and development must escape, so far as is possible, from the present economic difficulties".

Money was not the only thing the council had in mind. Its members considered that the links between research and education need to be tightened; and so the council added phrases to the law which imply that Chevènement and the minister for national education, M. Alain Savary, must get together quickly to work something out, before Savary presents his own law to parliament in the autumn.

The council also stressed regionalization, sharpening the definition of the proposed "regional consultative committees" on research and technology. It suggested words guaranteeing the mobility of personnel, and, in particular, emphasized the use of the French language in science. The use of French is "a fundamental objective" said the council, but admitted that it would be difficult to achieve. The research law should define a precise strategy, and the ministry should keep the council informed of progress on this issue, the council requested.

The broad support of the council for the research law will be welcome and significant, because the council is an important — if unusual — constitutional body. Its 200 members are drawn from all walks of life, but particularly from the working classes (as 140 members are supported by trades unions); and it gives its opinion and advice on such matters as are referred to it by the prime minister, or on matters that it chooses to study on its own behalf. Its advice is usually taken before major bills are put before the National Assembly, and although the advice is not binding, it would be politically inept to ignore it. Moreover, since the unions are strongly represented on the council, and since the same unions have a strong influence on the present socialist government, the council might be thought to have more weight than it had in the past.

The next ports of call for the draft law will be the Council of State (to check legality), the Council of Ministers (for final political approval by the government), and ultimately — perhaps by early summer — the National Assembly for parliamentary debate, and, if successful, passage into law.

Robert Walgate

eighteen months ago backed away in the face of a flood of national publicity from a proposal that it should share the equity in a company being set up by members of its department of biology.

Both university and industry representatives agreed that research agreements should require the minimum amount of secrecy, and that in general university scientists working with industry funds should retain full rights of disclosure on their research results.

One controversial topic which was discussed at the meeting was how to avoid potential conflicts of interest when a university scientist is involved with both university and private research teams working in closely related fields. The participants agreed that universities should be encouraged to draw up explicit conflictof-interest rules, and also that any research agreements with private companies should not "impair the education of students, interfere with the choice by faculty members of the scientific questions or line of inquiry they pursue, or divert the energies of faculty members from their primary obligations of teaching and research".

There was apparently more disagreement on whether it was appropriate to grant companies which sponsor university research an exclusive right to use the results of that research. Some participants argued that this was going too far, and that although the company should be offered a royalty-free licence, the patent should also be offered to others at the same time. Some argued, however, that non-exclusive licences would discourage companies from developing a new product, and that an exclusive licence to the results was an appropriate quid pro quo for research support. If exclusive licences were not permitted, companies might be reluctant to support university research, said President Derek Bok of Harvard University.

The meeting had been described as "Asilomar II", a reference to the 1975 meeting on the safety of recombinant DNA research which took place a few miles away from Pajaro Dunes. Just as the earlier meeting had provided the basis for the subsequent development of the safety guidelines on DNA research, Dr Kennedy said after last week's meeting that it had marked the beginning of an attempt to establish a national consensus on guidelines for collaboration between universities and industry.

News of the new guidelines, however, is likely to evoke just as much controversy as the original Asilomar document. Graduate students at Stanford have already organized a series of meetings to discuss their perspective on the impact of the new university/industry links (Nature 25 March, p. 283). The Pajaro Dunes meeting had defined the arena in which future debates between supporters and critics of close university/industry links will inevitably take place.

David Dickson

Polish science

Travelling again

A few Polish scientists are now arriving in Western laboratories and universities to take up exchange places arranged before the imposition of martial law. The delay in their arrival has not been due simply to the general confusion following the army takeover; they bring with them news of emergency regulations for scientific trips, which demand, in particular, that any such trip must be "closely in line with the aims of the foreign policy of the Polish People's Republic and the socio-economic and scientific policy of the country".

Under these emergency regulations, any proposed trip must be submitted for detailed analysis by the director of the institute or rector of the university where the applicant works. This has to take into account not merely the scientific purpose and the cost-effectiveness of the trip (especially as regards foreign currency), but also whether or not the applicant can be guaranteed "to represent the political interests of the Polish People's Republic". In particular, trips will not be authorized for persons "who have actively worked to the detriment of the state, or who have broken the regulations of the decree on martial law". (This clause, if strictly applied, would exclude scientists working in several institutes of the Academy of Sciences, the Swierk nuclear research institute, and other academic establishments which responded to the imposition of martial law by protest strikes.) Relatives of scientists already working abroad will not be permitted to join them, although it is not made clear whether this is simply due to the shortage of currency, or whether it is intended as a means of ensuring that the scientists concerned will return at the end of their tour of duty.

Preferential treatment, it seems, will be given to long-term exchanges, especially if they are concerned with research into subjects of particular significance for the Polish economy, and if the proposed visit is to a "leading" scientific institution abroad where the scientific and practical reward is self-evident. Visits planned under existing exchange agreements and contracts for visiting lecturers will also be given a more favoured status, while the officials of international scientific societies, and academics invited to take the chair at international congresses and symposia (if not otherwise disqualified) should be enabled to travel "in order to facilitate the participation of Polish scientists in leading international scientific events".

Students, on the other hand, are to have their applications "delayed", except for students studying at universities in socialist countries on the basis of international agreements, and students sent abroad by the Ministry of Science, Higher Education and Technology for one- or two-semester "short courses". The same delaying process is to be applied to researchers wishing to go abroad to collect archive material, consult with their colleagues on "non-priority" matters, or to take a "passive part" in an international conference.

Vera Rich

Universities in industry

Imperial goals

Imperial College in the University of London has set up a manufacturing company with venture capital. The college, which is providing the facilities for the new company, will share the equity equally with Technical Development Capital Ltd (TDC), a subsidiary of the Finance for Industry Group, which is putting up £400,000 as initial investment. Imperial Biotechnology Ltd, as the new company is called, will use the college's pilot fermentation plant to manufacture purified enzymes, proteins and specialized fermentation products. These will be used for the chemical and mainly pharmaceutical industries.

The new company is primarily intended to provide a solution to the college's recent difficulties in funding the fermentation plant, which is housed in the biochemistry department. Although the plant had been earning contract money, the college could no longer make up the running costs out of central funds. It is hoped that the new company will be at least self-financing, eventually achieving a turnover of a few million pounds per year.

About twenty staff working with the fermentation plant will be transferred from the college payroll to that of the new company. They will retain their existing commitments to teaching and research. Management of the plant, however, will be transferred from Professor Brian Hartley, head of the biochemistry department, to a new management team appointed by TDC. Dr Trevor Langley, formerly with Whatman Biochemicals Ltd., has been made managing director of Imperial Biotechnology Ltd.

Professor Hartley, a critic of the government's unwillingness to support more biotechnology posts in British universities, plans to use some of the money released by transferring staff to the company payroll to establish two new academic posts in the Centre for Biotechnology which he is at present establishing.

Imperial Biotechnology Ltd will operate much as any other company, using profits to finance expansion. It will, however, benefit from its position on the college campus by maintaining close links with research. Professor Hartley, who has links with the Swiss company Biogen, will also be scientific adviser to the new company. He sees no conflict of interest.

Judy Redfearn

Biotechnology

Looking ahead

Bethesda Research Laboratories (BRL), the Maryland company that shook the US biotechnology industry by announcing that cash-flow problems had made it necessary to make redundant more than a third of its 450 staff, seems — at least temporarily — to be out of the woods.

Company officials are confidently predicting that, despite the setback to its expansion plans, BRL will double this year the \$10 million sales which it achieved in 1981, thus maintaining the pattern of geometrical growth that the company has managed since its foundation in 1976. Comparable sales increases are being talked about for 1983 and 1984. Private investors in BRL have endorsed the line being pushed by Mr Stephen Turner, BRL's founder and president, that "leaner is fitter" - the argument, ironically, that is being used by President Reagan's science adviser, Dr Jay Keyworth, to justify cuts in federal support for research and development.

The company has just announced that it has been able to raise an extra \$5 million from its original investors, many of whom are based in Europe. According to Mr Turner, this should be enough to provide a stable base for a steady expansion of its product range, originally focused on restriction enzymes but subsequently expanded to include other biological

products such as monoclonal antibodies as well as diagnostic screening kits and a nucleic acid analyser.

Mr Turner blames the company's recent difficulties on the rapid drying up of venture capital in the United States in the past six months. Last year, anticipating that it would have little difficulty in raising an anticipated \$40 million through a public stock offering, the company laid the groundwork for an ambitious expansion programme, dividing its research products division into separate molecular biology and biological chemistry sections, and significantly increasing its research staff.

In recent months, however, a declining inflation rate and new tax incentives have taken some of the glamour out of venture capital dealings as an alternative to other, more traditional forms of investment. In addition, much of the available venture capital is said to have been soaked up by some of the early public offerings — such as that launched by Cetus a year ago, which managed to raise over \$100 million in the largest new issue ever experienced by Wall Street.

As a result, many of the small biotechnology companies launched in the past two or three years are experiencing severe difficulties in raising the capital they need to keep going, a situation that Mr Turner believes could last for at least another year. Several industry analysts expect that up to one half of the companies could disappear within the next few years, some being absorbed into larger

Rorvik versus Bromhall

Washington

Hailed in 1978 on the book jacket as "the scientific breakthrough of the century", David Rorvik's book In His Image: The cloning of a Human was declared by a Philadelphia judge the same year to be "a hoax and a fraud". Rorvik returns to a Philadelphia court next week when Dr Derek Bromhall from the University of Oxford, who claims that his research was misused, will sue for damages against Rorvik and his publisher Lippincott and Co. (now owned by Harper and Row).

Mr Allan Friedman, Bromhall's lawyer and an associate of the firm Raynes, McCarty, Binder and Mundy, claims that Lippincott showed "reckless disregard for publishing ethics". He says that his client's case turns on two issues. The first is the claim that Rorvik invaded Dr Bromhall's privacy by quoting his cloning techniques in the text and by mentioning his name in the book's footnotes as a "personal communication" without approval. The second claim is that Mr Rorvik fraudulently acquired an abstract of Dr Bromhall's doctoral thesis by representing himself as a scientific researcher preparing a project on mammalian cloning.

Mr Rorvik's attorney, Samuel Klein of Kohn, Savett, Marian and Graf, claims that Bromhall's information did not include "novel and unique" concepts, that the thesis was already on microfilm and that a summary had already appeared in the scientific literature (*Nature* 258, 719; 1975). Furthermore, he adds, there has never been disagreement over the fact that all references to Bromhall's work were fully accurate.

Mr Klein also plans to argue that in the single letter written by Mr Rorvik to Dr Bromhall, his client identified himself as a freelance journalist and that Dr Bromhall's presentation of his nine-page abstract was unsolicited. There was no oral communication between Rorvik and Bromhall at the time of this correspondence.

The trial promises to become heated, for neither party plans to settle out of court. Mr Klein also says he will question Dr Bromhall's wish to keep his distance from the book in light of the fact that Dr Bromhall "voluntarily associated himself" with the film Boys of Brazil, which portrayed the cloning of multiple Hitlers. But Friedman says that Bromhall's voluntary association with a film presented as fiction is very different from an involuntary association with a book whose author claimed it to be non-fiction.

The trial is expected to last several weeks and many scientists will make appearances for both sides.

Michael D. Stein

corporations, others filing for bankruptcy or actively seeking mergers. BRL claims to be in a stronger situation than some of the other companies since it is already selling products on the market.

"The days of over-expectation and pure speculation are behind us and the time has come to fine-tune operations and get down to the business of running a business," says Mr Turner. "That's what we have done at BRL."

Similar sentiments are expressed by Frederick R. Adler, a New York lawyer who has helped to set up a number of high technology companies in the computer field, and who has recently been appointed to BRL's board of directors. "The computer industry taught us that business can successfully serve science. We're now translating that lesson to biotechnology", he says.

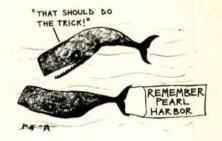
David Dickson

Sperm whale catch limits

No decision

A head-on clash between conservationist countries and Japan, the main whaling nation, was averted on Thursday when a special meeting in the United Kingdom of the International Whaling Commission (IWC) agreed to defer a decision on sperm whale catch limits until the annual general meeting in July. The conservationist countries abandoned their attempt to impose a total ban on sperm whaling after the scientific committee failed to produce a conclusive report.

Catch limits are usually set at the annual meeting of IWC. Last week's special meeting was convened because the scientific committee had not been able to offer unequivocal advice to the July 1981 meeting. Earlier this year, 32 scientists from 12 countries met in Cambridge to analyse sperm whale stocks. Computer tests were run using two rival models, one backed by the Japanese, the other developed by the International Institute of Environment and Development (IIED).



All except the Japanese scientists at last week's meeting supported the IIED model. However, the committee failed to offer any definite advice on catch limits. According to IWC criteria that stocks should be protected when they fall below ten per cent of maximum sustained yield (the maximum number of whales that can be harvested on an infinite basis without a decline in population), the IIED analysis indicated

that males should be totally protected, but a catch of 831 females could be permitted. But the analysis also indicated that even with a zero catch the stocks would continue to decline for several years. The biological reason for this is that sperm whales are polygynous so that the past removal of breeding males, favoured by the whalers because of their large size, has resulted in a decline in the pregnancy rate of the stock.

Therefore, the Japanese argue that their current quota of 890 would make little difference to the sperm whale population, particularly since the number of exploitable whales was estimated by the IIED team to be around 200,000. But conservationist countries are keen to see a zero quota, pointing to the predicted decline in population with no fishing at all.

The inability of the special meeting to come up with catch limits was not so much the result of scientific equivocation as a political move to produce a compromise. The conservationist countries feared that had they enforced a quota unacceptable to the whaling nations, Japan would have exercised its right to object and both the procedure for cooperation and the whole credibility of IWC would have been seriously damaged.

The annual meeting of IWC in July may be faced with pressure for a ban from the conservationist countries, which will be in the majority. If Japan refuses to compromise and registers an objection, the conservationists would then have to try to apply the ultimate sanction — a US ban on Japanese fishing in its waters.

Jane Wynn

Environmental law

Count the risks

Washington

A subcommittee of the House of Representatives has taken a first step towards building scientific risk analysis into the federal government's regulations on environmental and safety hazards. A bill passed by a subcommittee of the Science and Technology Committee would establish a new programme under the White House's Office of Science and Technology Policy (OSTP) "to improve and facilitate" the use of risk analysis in regulatory decisions. The bill will now go to the full committee, whose chairman, Mr Don Fuqua of Florida, supports it.

The Reagan Administration is less welcoming, feeling that the goals of the proposed law could more simply be attained by rationalizing new and existing regulations. But according to OSTP, the Administration will probably find the bill "acceptable" as long as certain amendments are made.

Various members of the Administration argued in their testimony to the subcommittee that they have already taken steps to introduce a more "rational" approach to regulation. Last February, for example, President Reagan put out an executive order requiring all proposed new regulations to be accompanied by a costbenefit analysis demonstrating that they represented the most cost-effective way of reaching their declared goals. The White House has also announced that the Inter-

agency Regulatory Liaison Group, established under President Carter to coordinate actions by different agencies, would be reconstituted as a new committee under the chairmanship of the President's Science Advisor, Dr Jay Keyworth, with the task of improving the scientific basis of regulatory decision-making.

Such changes have not satisfied the members of the House committee. Thus the new bill would assign to OSTP the specific role of establishing a mechanism for coordinating the risk analysis programmes among the participating federal agencies, primarily the Consumer Product Safety Commission, the Food and Drug Administration, the Environmental Protection Agency and the Department of Labor's Occupational Safety and Health Administration. And the National Science Foundation would be required to draw up a programme of research to make the use of risk analysis more effective. In addition, the various federal agencies would each be required to undertake a "prototypical risk analysis study".

Various powerful lobby groups, representing for example the agricultural or the nuclear power industry, have long been pushing for revisions to regulatory decision-making along these lines. The bill is therefore unlikely to lack supporters if it is sent by the full committee to the floor of the House of Representatives. But it is also likely to generate substantial opposition. Many Democrats, particularly those with close links with the labour unions, see attempts to "rationalize" regulation as a smokescreen to legitimize less stringent health and safety controls.

The Administration is likely to stand aside from any such debates. Although it claims to have made significant progress behind the scenes in eliminating unnecessary or redundant regulations, it is becoming wary of taking highly-publicized actions that might be interpreted as "antiregulatory" given the apparent continued public support for strict environmental and health and safety controls — and the importance of the congressional elections later this year.

In a related effort to reduce the impact of federal regulations, the US Senate last week approved a proposed new bill which would give Congress the power to overturn regulations being proposed by any federal agency. The bill is being resisted by the Reagan Administration, which sees it as an attempt by Congress to place limits on the flexibility of the executive branch. It has also received strong opposition from environmental and labour groups, who argue that it would provide vast scope for individual companies and industries to lobby Congress to prevent certain regulations from coming into effect. Supporters of the new ruling, however, argue that regulatory agencies frequently exceed their congressional mandates, and that checks are now needed to limit their autonomy. **David Dickson**

Join the queue for monoclonals

St Louis

The Monsanto Corporation of St Louis, Missouri, has made a multimillion dollar agreement with nearby Washington University to produce monoclonal antibodies, the second such agreement the university has made with the chemical industry in the past six months.

Monsanto says it has agreed to put \$1.8 million into research projects at the university during the next three years. No contract has so far been signed between the university and Monsanto but the deal is expected to be similar to that made with Mallinckrodt Inc., also of St Louis. Last September, Mallinckrodt committed \$3.9 million to monoclonal antibody research at Washington University, the largest industry agreement ever made with an American university for this type of research.

Mallinckrodt's three-year commitment is a major one for the company, which is economizing in other areas. But despite uncertainties about patents, monoclonal antibodies hold out enormous commercial promise. Dr Thomas O. Oesterling, Mallinckrodt's

vice-president for research and development, says his company's primary interest is in *in vitro* diagnosis, where monoclonal antibodies allow much improved standardization in diagnostic kits, but it is looking at other uses. "A monoclonal antibody could carry an isotope for imaging to a target cell for *in vivo* diagnosis", and from there it is "a logical extension to therapy".

The contract between Mallinckrodt and Washington University provides for the university to hold the patents for useful products of the joint venture, but for Mallinckrodt to have the right to license them. The university's share of royalties is dependent on the success of the product on the market.

One aspect that is covered by the arrangement with Mallinckrodt is the right of publication. Individual investigators do not profit personally from an invention, although some of the royalties will be ploughed back into research in the individual's laboratory, but the individual does retain the right to publication, with the proviso that the company can screen for patentable material.

Karen Freeman

Making waves

Following hard on the heels of Amersham International, another "public" company — the Hydraulic Research Station at Wallingford, Oxfordshire — is to go "private" on 1 April, with Dr John Weare as managing director and chief executive.

All of the present staff will be seconded from the civil service to the company for one year, after which 80 per cent (about 200 people) are likely to be offered long-term contracts.

The transfer was not without its hitches. Staff at first resisted the move fearing that "privatization" would mean loss of government backing and lead to a lessening of the station's world-wide reputation. In fact, no drastic cutbacks are planned and the general feeling is that, freed from government constraints, the company will be better able to respond swiftly and competently to the needs of the civil engineering industry.

Sara Nash

Indian science education

Falling behind

New Delhi

In a major new exercise, Indian government circles concerned with science education are trying to find resources equivalent to \$55 million for the next three years to strengthen the science education base of the universities.

The government realizes that lack of support has allowed the university science system to run down and that this trend, if continued, may prove irreversible. The role of universities as advanced centres of teaching and research has been eroded by the rapid expansion in the number of institutions and students without a corresponding increase in the necessary facilities.

The Science Advisory Committee to the Cabinet (SACC) has long felt that the resources of the universities should be augmented as this would not only benefit universities but also assist the major scientific agencies of the country and industrial enterprises. The scientific manpower requirements of these agencies will be met by the universities. SACC recommended that central and state governments and industrial companies should combine to contribute about \$55 million to strengthen the science base of the universities. A detailed scheme is expected to be submitted to the cabinet for approval.

The Indian government also feels that the current pressure on the universities in terms of the enormous intake of science students should be reduced, and that equal opportunities should be provided for gainful employment for those who do not pursue careers in science after passing the school level examination. This system will

then provide the smaller number of science students with proper facilities. And as lack of instrumentation is a serious handicap for research in universities, the government is now considering stepping up instrument production.

It is widely believed also that the links of university research and national scientific agencies with public sector enterprises need to be strengthened. The present barriers between the two are impending the country's scientific and technological progress.

Sunil Saraf

•Speaking at the invitation of the Science Policy Foundation in London last week, Mrs Indira Gandhi reasserted her commitment to the strengthening of Indian science and technology. Without internal strength, she said, the governments of developing nations cannot withstand the tendency of some nations to use the transfer of technology as an instrument of foreign aid. "We have not got out of one empire to get into another" was her reply when later questioned as to her country's relationship with the transnational corporations.

Self-reliance is also necessary, Mrs Gandhi said, because the problems of India are increasingly different from those of the developed nations. India needs to curb population growth, to find ways to grow crops in soil of low moisture and to prevent cholera and tuberculosis, not cancer and heart disease.

At the same time it is of immense benefit, even a necessity, for India to continue its own atomic energy, oceanographic and space programmes. "Our space effort is important for education and communication and deeper knowledge of the monsoon, which rules our economic calendar."

While emphasizing the unacknowledged, reverse economic aid donated by emigré Indians to "Western" scientific progress, Mrs Gandhi said that opportunities for intellectually challenging work must be increased to keep talented Indian scientists in the services of their own people

Peter Newmark

National Research Council

Changes ahead

Washington

The US National Academy of Sciences is carrying out a major reshuffle of the complex organizational structure of its National Research Council (NRC). The purpose, according the academy's new president, Dr Frank Press, is "to meet the opportunities ahead of us, to promote greater efficiency in our ability to respond to governmental requests and to meet changing economic circumstances".

NRC, the operational arm of the academy, provides advice to and carries out research on a contract basis for the federal government and other

organizations. Since 1973, it has operated through four assemblies — each based on a single discipline or set of disciplines — and four multidisciplinary commissions.

This arrangement was introduced to improve NRC's ability to tackle policy questions that frequently cross disciplinary boundaries. In practice, however, there has frequently been confusion over the relative responsibilities of the various assemblies and commissions — a situation which Dr Press hopes the new structure will avoid.

Three of the assemblies will therefore be merged with three of the commissions. The Assembly of Behavioral and Social Sciences will be merged with the Commission on Human Resources to become the Commission on Behavioral, Social Sciences and Education; the Assembly of Engineering will merge with the Commission on Sociotechnical Systems to become the Commission on Engineering and Social Systems; and the Assembly on Mathematical and Physical Sciences will join with the Commission on Natural Resources to become the Commission on Physical Sciences and Resources.

The future of the fourth assembly, the Assembly of Life Sciences, some of whose work currently overlaps with that of the Institute of Medicine, will be discussed at the next meeting of NRC's governing board which takes place on 3 April. Any changes to the constitution of the Institute of Medicine would have to be approved by its members.

The fourth commission, on international relations, will be transformed into a new Office of International Affairs, whose executive director will be Dr Victor Rabinowitch. The activities of the office will be overseen by a panel comprising the foreign secretary of the academy, Dr Walter Rosenblith, the foreign secretary of the National Academy of Engineering, the president of the Institute of Medicine, and Dr Rabinowitch; this panel will also undertake policy formulation for the whole of the academy complex.

The reorganization is part of a broad attempt by Dr Press to streamline the academy's activities and enable it to play a more active part in Washington science policy debates. One example of this new approach is a panel which is being set up under the Committee on Science and Public Policy (COSPUP) to examine the implications of the closer links being drawn between US universities and the Department of Defense.

The panel will examine a range of issues raised by this enhanced relationship, from the application of export controls to unclassified research to broader impacts on academic freedom. Funding for the study has already been promised by the Defense Department itself, the National Science Foundation and the American Association for the Advancement of Science; NAS is seeking additional funds from outside sources.

David Dickson

CORRESPONDENCE

The coal of today

Sir — The content of the leading article "A strategy for coal" (Nature 25 February, p.636) indicates a view based on out-dated knowledge of the coal industry. The economic arguments used ignore, for example, the fact that British coal is the most competitive in the European Community. The latest published coal production cost comparisons (for 1980) are:

	£ per to
Belgium	61
France	45
West Germany	44
UK (1980/81)	35

The article mentions the design of microcircuits as if this was a world apart from coal mining. In fact, Britain's technologically advanced coal industry extensively uses computers, microprocessors, transducers, lasers and other modern aids. There is obviously no future in the microtechnology unless there are industries like coal to use the products. The National Coal Board and private enterprise manufacturers have successfully designed and introduced computerized control and monitoring systems for colliery operations and these are creating considerable interest overseas.

Longwall mining technology was pioneered in Britain and equipment sales remain significant export earners — and have resulted in record-breaking colliery performances in the United States, South Africa and Australia. There are many other examples showing that our indigenous coal production is serving Britain well — and it will need to do so in the future. Incidentally, the Australian exports mentioned in the Nature article include a high proportion of opencast coal which is also profitably produced in Britain. ROBERT HUNT Dowty Group, Cheltenham, UK

Doves in false garb

SIR - Having read more than once your leading article of 18 February (p.542) under the condemnatory title above, I am still puzzled that you provide no evidence, by way of argument, to justify the statement "The claim by the anti-nuclear movement of professional support is mostly a sham". The occasion you were commenting upon, the Conference of Professions for World Disarmament and Development, convened under the auspices of the World Disarmament Campaign, made no claim to speak officially for the major professional societies. But the attendance of several hundred professional people, and the rapid growth of specialist bodies such as Scientists Against Nuclear Arms (SANA), Medical Campaign Against Nuclear Weapons, and Teachers for Peace suggests that there are many who do agree in general terms that "the professions as such have a responsibility to alert the general public to the great issues that confront society". You seek to deny this, yet you accept in the very next sentence that "there is a sense in which professional people, acting individually, may be held to shoulder an extra responsibility for giving wider currency to their conclusions about important issues on which they have some special knowledge".

Why the emphasis on acting individually?

Why do you consider it "not respectable" for scientists of different disciplines to come together through SANA to pool their varied expertise relevant to the effects of nuclear war on Britain, and then to make known their conclusions based on this special knowledge? Why try to belittle the work of the Medical Campaign Against Nuclear Weapons and the Medical Association for the Prevention of War, who point out to their colleagues that nuclear war would be a medical catastrophe beyond reach of any cure, so that it becomes a professional duty to seek its prevention?

You complain that the major professional bodies "are needlessly indifferent to important public issues well within the spheres in which their professional competence could command respect". Yet you condemn, by implication, the work of the British Medical Association to study the medical effects of nuclear war, and the efforts within the teaching profession to direct attention to the role that education plays in forming public attitudes to other countries and to conflicts within and between countries. Do you not accept, for your own profession of journalism, some responsibility for the way in which the press and television treat major issues such as nuclear war and thus influence the formation of public opinions?

CHRISTOPHER MEREDITH
(Secretary, Scientists Against
Milton Keynes, UK Nuclear Arms)

SIR — The leading article on the meeting of professional groups concerned with supporting nuclear disarmament makes a number of astonishing assertions (*Nature* 18 February, p.542). For instance it seems to question the right of the medical profession to express an opinion on the issue, claiming that "Physicians have been consistently indifferent to the quality of health care (in Britain)". This suggestion is as insulting to the medical profession as it is unfounded.

The health care professions should and do have a special knowledge of the medical effects of nuclear weapons as have other professional bodies, such as architects, specialized knowledge of such matters as affect their sphere. It would seem entirely understandable, and we would submit entirely proper, that such organizations should undertake to publicize as widely as possible the full effects of the use of nuclear weapons.

The rapidly expanding Medical Campaign Against Nuclear Weapons, and no doubt the other professional groups represented at the meeting discussed in the article, reflect the widespread concern over the proliferation of nuclear weapons and the apparent increasing risk of their use. In the United States, professional bodies voice similar concerns. Thus a recent report of the American Medical Association stated "There is no adequate medical response to a nuclear holocaust".

Doctors and other professional organizations not only have a right but a duty to speak out on the subject of nuclear weapons and nuclear warfare. They do so in order to bring the facts to the attention of their colleagues, politicians and the general public and not, in the remarkable words of the leader, to capture any "mediaeval mystique".

M. HARTOG, J.H. BAUMER, P.J. FLEMING, M.J. HALL

University of Bristol, UK

SIR — The views expressed in your leading article entitled "Doves in false garb" (*Nature* 18 February, p.542) were put forward provocatively enough to require reply.

You have allowed that "there is a sense in which professional people, acting individually, may be held to shoulder an extra responsibility for giving wider currency to their conclusions about important issues on which they have some special knowledge". Why must they be "acting individually"? Why, that is, when medical people act together, do you decry this as their "attempt to invest their legitimate causes with spurious authority . . . a deceit"?

Medical people certainly have no entitlement to speak with any lofty authority. But we have seen people die of burns and crush injuries, and have had to repair terrible lacerations. There is always at such times the question "why need this have happened?". and physicians, surgeons and general practitioners alike bear, or should bear, prevention constantly in mind. For epidemiology prevention is the raison d'être. It means searching for any predisposing causes of disease or injury and proclaiming them when they are found. For many of us it is no longer tenable to act in this way where, say, chemical carcinogenesis, dietary causes of disease or accidents in the home are concerned, but to remain mute on the dangers of nuclear detonations. Psychiatrists especially are left with few illusions about human fallibility. STEWART BRITTEN London, UK

A final word

SIR — "The creationists may have lost the battle in Arkansas but they are unlikely to abandon the war on which they are engaged" (Nature, 14 January, p.85).

Your journal has, for several months, lent itself as a battleground for this verbal brawl by continuing to publish letters from both sides, many of which do not (and cannot) contain any elements of scientific reasoning. This correspondence has degenerated into a discussion of the Bible as a source of artistic inspiration (A.J. Hollin, *Nature*, 18 February, p.548) and such fine points as the positioning of a comma in Luke's gospel (F.W. Cousins, *Nature*, 11 February, p.452). These authors clearly have a sense of the absurd.

Nature is a journal with a large and mixed audience which serves not only to publish important scientific findings, but also to inform scientists about political issues which are of particular interest to them. The Arkansas trial was clearly one such issue. That trial is now over, and scientists wishing to continue the discussion should now look to do so from a platform where they might be better able to inform and enlighten the general public. Does Dr Salthe (Nature, 11 February, p.452) really need to point out to a scientific readership the folly of the statement that "The scientific community does not consider origins of life a part of evolutionary theory"?

Serious discussion of mechanisms involved in evolution has a place in this journal.

Advocates of God and *Genesis* should air their views elsewhere.

JANE CALVERT University of Alabama, USA

This correspondence is now closed — EDITOR

An end to the search for new drugs?

M. Weatherall*

THE discovery of new drugs is taking longer and becoming less productive1-3. In the United Kingdom the average time between first publication (itself usually years rather than months after studies begin) and marketing increased from 6 years in 1965 to 10 years in 19784. In the United States the average time between filing an "Investigational New Drug" (IND) - in effect advising the authorities of an impending trial in man — and approval by the authorities of a drug for general use increased from about 3½ years in 1967 to 9 years in 19765. One British pharmaceutical company has found⁶ that between 1972 and 1978, the number of animals used in safety testing increased from 600 to 1,200 or more and that the number of man-days spent by trained staff on a single nonrodent study increased from 275 to 480 despite improved working methods which saved 85 man-days. Unpublished figures from the Wellcome Foundation show that the cost of studies necessary before clinical trial of a drug intended for long-term use in man rose between 1965 and 1979 by a factor of 3.2 for toxicology and 5.2 for pharmacokinetics, even after correction for inflation.

The money for this work has to be found somewhere. The more that is spent on bringing one drug into general use, the fewer the total number of drugs that can be studied and the more necessary it becomes to concentrate on common diseases where the prospects of a financial return are greatest. Drugs for rare diseases sometimes called "orphan" drugs - such as triethylene tetramine for Wilson's disease⁷ or L-5-hydroxytryptophan for myoclonus⁸, are not commercially viable and so must be prepared in a physician's laboratory and encapsulated in a hospital pharmacy7 if patients are not to be allowed to die.

It seems probable that financial pressures have already reduced the total research in the United Kingdom directed at finding new drugs. Cavalla⁹ lists nine companies which have closed their research and development groups, with redundancies or reorganization.

It can, of course, be argued that drugs are useless if not positively dangerous, and that the resources used in drug research would be much better spent on measures of more general benefit to the health of the community. There is a need to balance drug development against spending on

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community health, preventive medicine and relevant education. But as the funds come from different sources, striking a balance is necessarily difficult. Making drug development a government responsibility is not, however, an answer. A government concerned to live within its income from taxation would face the same problems as industry, and would perhaps be even more reluctant to undertake the risks of financial loss associated with speculative research, the problems of making orphan drugs available for uncommon but killing diseases and so on. Ultimately, health can be achieved and maintained only by work. The limit of health care is the amount of work which is put into it, and unless the work is unpaid, the limit is economic.

Meanwhile, until the balancing act is achieved and the public desire for medication¹⁰ abates, drugs will continue to be a major element in therapeutics and better drugs will be sought. No drug is without its hazards. The one prediction that can con-

evidence about the effects of drugs used by specialists in closely supervised patients. The effects may differ greatly when a drug is released for widespread and unregulated use. Information about the overall prescribing of drugs is of little help in assessing benefit, while the proportion of prescribed drugs actually consumed by patients is usually not known. The reporting of adverse reactions is patchy and biassed, and the fashion for expressing alarm about iatrogenic disease needs to be tempered by a careful appraisal of how the sufferers would have fared without the treatment.

In particular instances, thorough studies allow an assessment of risk. Thus immunosuppressive treatment, with azathioprine, chlorambucil or cyclophosphamide, is used to prevent rejection of transplanted kidneys. These treatments carry a 1.3% risk of tumour induction and 0.4% risk of mortality within the period of observation¹³. But this risk has been estimated only after ten years of study. It could not

Public demand for totally safe drugs has led to excessive, costly and misleading toxicity testing. Such testing drains the resources which have been available for discovering much needed new drugs. Better public understanding of the limitations of toxicity testing and the hazards of medication is sorely needed in order to improve the prospects for diseases still needing effective treatments.

fidently be made about any new drug is that it will have unwanted effects, and dangers if misused¹¹. Moreover, it is usually difficult to assess what contribution drugs have made to the reduction of ill health and prolongation of life, when sanitation and nutrition have improved at the same time. Yet in a variety of circumstances, drugs are unequivocally saving individual lives¹², as with the use of antibiotics in a wide range of acute infectious diseases, and of vaccines in the elimination of smallpox throughout the world and in the minimization of diphtheria and poliomyelitis in many countries. Replacement therapy prolongs life unequivocally: no one could seriously maintain that all diabetics could remain healthy without insulin. There is no doubt that some drugs are vitally necessary.

There is also no doubt that over a wide range of drugs, the evaluation of therapeutic benefit is extremely difficult. Difficulties arise when prolongation of life and easement of disability are the main expectations. Mortality statistics in more developed countries are good, but morbidity statistics are variable and, except for notifiable diseases, hard to come by. Clinical trials seldom extend over more than a year or so, and by their nature give

have been estimated when renal transplantation began. Had it been possible to know it, and had it been added to the operative risks and other hazards, would it have been a reason for not embarking on the procedure and would most of the people now living with transplanted kidneys have been allowed to succumb to their disease?

Drugs are tools which function by modifying the processes of life, and they cannot be labelled good or bad without considerable knowledge of the circumstances in which they are actually used. For many diseases, the drugs available now are clearly inadequate to restore health or even minimize discomfort and disability. Allergy, arthritis, cancer, cardiovascular disease, congenital abnormalities, psychiatric, neurological and a variety of tropical diseases all await effective treatments. There is a rapid growth in knowledge about the biochemical disorders and genetic background of many of these conditions, and either from such knowledge or by more empirical methods, new drugs continue to be found. But there is a drug lag in countries which have the most sophisticated regulatory arrangements14, and a virtual halt to the discovery of new drugs for poorer and less healthy nations 15

One reason for the decline in innovation may be the difficulty of devising suitable laboratory experiments for identifying drugs for the unconquered diseases. When a disease is due to a known infecting organism, compounds can be selected according to knowledge of the biochemistry of the organism, or empirically. Such compounds can be tested against the organism in vitro and in vivo, and the most effective selected for further study. The search may be long. but the route is clear. It has led to the whole range of chemotherapeutic drugs and antibiotics which have outstanding powers of saving human and animal lives. But this field is largely worked out, except for the tropical diseases. It will grow again in importance, as more drug-resistant organisms evolve. The resources for such discoveries are still an important part of the infrastructure of public health.

Drug searches

For diseases not known to be due to an infecting organism, some other clue is necessary to start the search for useful new drugs. When a particular substance is shown to be lacking, its replacement is indicated, as with L-dopa for Parkinson's disease16 and, earlier, in the various endocrine gland failures. New local hormones or mediators continue to be discovered, but it remains to be seen how extensive the practical therapeutic consequences will be of the great body of recent work on prostaglandins, leukotrienes and neuropeptides. There are advances to be made in neuropharmacology as the relationships between disorders of synaptic transmission and mental disease become understood. At present the field is confused and confusing, and hampered by the lack of reliable animal models for essentially human disorders. So, too, diseases involving some ill-defined immunological disorder rheumatoid arthritis^{17,18}, allergies of all kinds, perhaps multiple sclerosis¹⁹ — must either await biochemical understanding or rely on animal models justified in the main because they give positive results with agents already known to be effective in the human disease. This is a route to me-too drugs (not necessarily worthless) and to much waste of resources on false positives, but it is not likely to lead to great innovations in therapy.

The other major strategy for discovering new drugs is to pursue the detail of a physiological or biochemical system and to exploit its practical application. This is the natural course of academic scientists, and has led to such important agents as insulin and dimercaprol. In the past thirty years, however, the practical application by academic scientists of their discoveries has declined²⁰, partly because the resources now needed for development are completely beyond the range of university facilities and partly because increasing specialization within universities has reduced the cross-links between approp-

riate specialists, especially organic chemists with pharmacologists, and pharmacologists with clinicians21. The fundamental approach has been pursued more successfully in industry, notably in Hitchings' work²² on purine and pyrimidine metabolism and inhibitory analogues, which led to drugs as diverse as the antimalarial pyrimethamine, the antineoplastic agent 6-mercaptopurine, the immunosuppressant azathioprine, allopurinol for gout and the antibacterial trimethoprim. More broadly, all the developments of drugs associated with neuroeffector and synaptic transmission rest on the fundamental work initiated by Dale at Wellcome Research Laboratories, extended in countless other institutes and applied to practical ends by many pharmaceutical manufacturers.

A major development of this kind is necessarily a long haul and involves extensive investment of resources in a concept, the therapeutic merit of which can be judged only at the end of the road. This kind of investment requires circumstances of prosperity and reasonable security, and is hindered if the always limited resources for research are frittered away in unimaginative and invalid routines.

In a reasonable world, the increasing difficulties of discovery and the seriousness of the incurable diseases may be seen as good grounds for a more tolerant attitude to the hazards of therapy. Desperate ills need desperate remedies, but the wish for completely safe remedies has had and is having the gravest effects on drug discovery²³. The problem of finding valid laboratory models for the unsuspected toxic effects of drugs is just as difficult as the problem of devising methods for detecting therapeutic potential.

The major tragedies which have created public alarm and fear and which have led to the condemnation of drugs have arisen in special circumstances (pregnancy and thalidomide; consumption of amine-rich foods and monoamine oxidase inhibitors: Japanese life and clioquinol24) or have been due to unexplained sensitivity in a minority of patients (triparanol25, practolol^{26,27}, clozapine^{28,29}). Practolol, a β -blocking agent introduced for the control of cardiac arrhythmias and subsequently found valuable also in hypertension, is particularly notable for the thoroughness with which its toxicology was studied in animals, to the satisfaction of registration authorities. Nevertheless, adverse reactions, some serious, developed in a small proportion of patients who received the drug; the ill-effects included skin rashes, impairment of secretions and, most gravely, corneal damage sometimes leading to impairment or loss of vision (the "oculomucocutaneous syndrome").

None of the recognized laboratory procedures gave warning of the serious clinical syndrome nor, even with hindsight, has a procedure been discoverd which would have predictive value. Other "idiosyncrasies" are associated with particular genetic factors, as with reactions to suxamethonium³⁰, primaquine³¹, penicillamine³², sodium aurothiomalate¹⁸ and hydralazine³². When much more is known about individual biochemical variation and its genetic control, it may be possible to predict that particular drugs will be hazardous to particular individuals. But the animal models of orthodox toxicity testing give no basis for such subtle predictions.

The unsatisfactoriness of predicting adverse effects in humans from animal experiments has been well known for a long time³³⁻³⁶. Positive findings in animal experiments are also not always reliable evidence of harm to man. Usually, positive findings in experiments in animals are grounds for not giving a compound to human beings at all, but sometimes some toxic effect in animals is discovered after a drug is well established in clinical use. Furosemide, a well tolerated and valuable diuretic in man, causes severe liver necrosis in mice³⁷ because of a metabolite which is not formed to a serious extent in humans 38. The nucleoside 6-azauridine is tolerated for relatively long periods for cancer chemotherapy in man: in dogs, smaller doses than are acceptable in man produce potentially lethal bone marrow depression in 7-10 days39. Intramuscular injection of iron sorbitol causes sarcomas at the site of injection in rats and rabbits⁴⁰. The implication for human therapeutics appeared serious⁴¹; but the nonspecificity of the effect in laboratory rodents was recognized42 and, nearly 20 years after the original observation in rats, only eight published cases could be found of tumours after intramuscular iron injection; their variety did not suggest a common origin from the injected material⁴³.

Species specificity

Every species has its own metabolic pattern, and no two species are likely to metabolize a drug identically44. Small differences in the rate of conversion of drug to inactive, or to toxic, metabolite can have large effects on the concentration of active substance at the point of action. Most experiments to seek toxic effects in whole animals involve oral administration: differences in diet, gut physiology, rate of passage and liver enzymes raise serious questions about the relevance of findings in rats or mice to man. Compounds which are not absorbed in laboratory animals are not, with minor exceptions, ever tested in man. Nobody knows how many drugs, which would be useful in man, have been lost in this way. Similarly compounds toxic in laboratory animals at doses near the predicted therapeutic level do not receive trial in man, so it is never revealed whether they would actually have been harmful in man. Thus we lack the evidence of the false positive element in animal toxicology studies39, so it is easy to give more weight to such studies than is justifiable.

The procedures45 which became

established after thalidomide whereby large numbers of animals were fed large amounts of drug for a long time, have become ritual "routine tests of limited value and governed by regulations rather than by rational thought". Reports on the results are submitted to regulatory bodies in confidence and seldom published. The experiments are rarely repeated by independent workers: nobody is motivated to find the very considerable resources needed to do so.

Supplementary tests — for effects in pregnancy and on fertility, for carcinogenicity, for mutagenicity - grow in number but not in validity, and the search for quicker and cheaper tests, notably using mutagenicity in bacteria in vitro as evidence of carcinogenicity in vivo, has added to the obstacles, uncertainties and expense of developing drugs. As with the primary toxicity tests, positive findings are a bar to further progress, and to validating the test, so that the procedures acquire a possibly unmerited aura of respectability. The greater the number of tests, the greater the chance of a false positive and the loss of a useful drug.

There are good reasons for special caution in applying results of experiments in vitro to conditions of practical use, especially when massive concentrations are used. In mutagenicity tests, for example, considerable adjustment of experimental conditions appears to be necessary to obtain a positive result with a chosen compound. Ashby and Styles⁴⁷ list 14 factors which can be varied in, and influence the outcome of, the Ames test, which is widely accepted as a standard basic procedure. By variation of any one or more of these factors, the sensitivity of the test can be altered, sometimes by as much as 400-fold. For the innocent who wish only for a black or white answer, the outcome can be readily changed from positive to negative or vice versa.

In vitro reliability

In other words, there is a considerable scope for adjusting the test to give the required answer with any specified compound, and it is a great help in selecting test conditions to know what answer is wanted. Ames and Hooper⁴⁸ observe that "using rat liver homogenate as a model for a rat's metabolism of foreign chemicals is a reasonable first approximation". But, in vivo, compounds are metabolized and excreted, either unchanged or as metabolites. It is as important to mimic the mechanisms of inactivation as of activation if misleading results are to be avoided. The actual concentrations to which DNA is exposed in real life are quite unpredictable from concentrations produced in such experiments in vitro. The use of human instead of rat liver would add marginally to the predictive value of such bacterial tests for human mutagenesis. The addition of further enzyme systems might be better still, but would not overcome the problem of relating the tests to real life concentrations and duration of exposure.

Indeed, unless mutagenicity in vivo differs from all known pharmacological effects, it will be affected by the concentration of the agent to which the DNA is exposed and by the length of time and by the frequency of exposure, none of which can be properly assessed from data in bacteria in vitro. Even if mammalian DNA is damaged in the same way as the DNA of bacteria in a test situation, repair mechanisms are well known in mammals49. and may annul any damage so caused. The extent of their protective role has yet to be established. But no compound has been proved to be a mutagen in man and, 35 years after the massive radiation of the people of Hiroshima and Nagasaki, no deleterious heritable mutations have been recognized, so the protection afforded by repair mechanisms has had a substantial trial and appears to be pretty good.

Finally "the" correlation between mutagenicity (in bacterial tests) and carcinogenicity in humans is anything but simple. Many more carcinogens are known in rats than in humans and demonstration of carcinogenesis in rats sometimes depends on notably large and repeated doses of the substance under test⁵⁰. The importance of a carcinogen, as of any other toxic or therapeutic agent, depends on the concentration and persistence of the substance at the site of action, or more roughly on the dose and duration of exposure. Disregard of quantitative considerations is the first step — and it can be a very long step indeed — towards nonsensical prediction and the abandonment of useful substances on ridiculous grounds50

The problem remains of deciding what weight to attach to results of ominous but uncertain significance. The quicker and cheaper a test, the greater the number of substances on which suspicion may be cast. The list already includes the essential antimalarial drug chloroquine51, and such more familiar substances as caffeine52, tap water⁵³ and oxygen⁵⁴. Common sense prevents alarm about oxygen, which does, after all, damage the lungs and central nervous system of adults⁵⁵ and cause blindness in premature infants56 at only five times the normally accepted concentration. Indentification of a hypothetical carcinogen or co-carcinogen among the solids dissolved in some samples of tap water is less certain to lead to a major advance in public health than to promote expensive but nugatory research, paid for by charity or the public purse.

Coffee drinkers are no more likely than smokers to be diverted from their habits. The epidemiological evidence of association between coffee drinking and carcinoma of the pancreas⁵⁷ requires further investigation to establish whether one causes the other or whether both arise from an unidentified cause, but such an investigation will at least relate directly to a human problem. Will regulatory

authorities become concerned with chloroquine regardless of its place in the control of malaria? The regulatory authorities mostly operate in the rich countries, which can afford them and which are relatively untroubled by malaria. If chloroquine were banned as a mutagen in any western country, would its further supply to less developed countries appear to be morally responsible? The difficulties of preventing "character assassination" of a drug are great, witness the problems of phenacetin⁵⁸, metronidazole⁵⁹⁻⁶¹ and Debendox⁶², but the resulting losses to human health may last for ever.

What really matters is what happens in everyday life. Recognition of the fallibility of laboratory studies has led to a welcome demand for surveillance of the effects of drugs in ordinary use23. However, it is very difficult to measure the incidence of adverse drug reactions^{63,64}, most of which are infrequent and occur in patients who are usually the recipients of several different drugs - and who may not necessarily consume the drugs prescribed for them. Naturally, a drug suspected of causing a reaction is withdrawn and many reactions subside. To give the suspected drug again so as to discover whether its toxic effect is reproducible, is hazardous and unethical, so evidence of individual cases is of very poor scientific quality.

Simplify regulation

Various schemes of surveillance have been proposed^{65,66}, by which it is hoped that quantity of evidence will overcome the weaknesses of separate observations. The accumulation of much data is essential. and the costs are substantial. If the price of drugs is not to rise, savings must be made elsewhere64. One obvious area is in preclinical evaluation and its administrative costs. The recent simplification in requirements in the United Kingdom for a clinical trial certificate⁶⁷ are therefore welcome, though the financial savings will be modest. Studies of post-marketing surveillance are desirable, but if expensive schemes add to the economic barriers of discovering new drugs, they may yet achieve more social loss than benefit.

A basic difficulty lies in public opinion, and in the public belief that drugs can be, and ought to be, completely safe. Public conception both of the probability and of the severity of a risk is complex and not well understood. A single large-scale tragedy evokes much fear, as after an air crash or the sudden revelation that many deformed babies had been born after the use of thalidomide, whereas a continuous series of small tragedies, as in road accidents, is treated apathetically^{68,69}. Most people understand that a surgical operation carries a finite risk, and face with more or less equanimity the remote possibility of death on the operating table or some other ill-fortune. But few appreciate a similar remote risk from consuming a drug, and the majority are correspondingly shocked

when such an event occurs. The public view might be expressed in such words as "everything that could have been done to make a drug safe should have been done; even if tests are not very reliable, they had better be negative". A better understanding of the factors which create such views is needed if real safety and perceived safety can both be attained. Comparisons of the risk of taking the drug with the risk of omitting it from treatment are hard to make and harder still to present convincingly. And, ironically, it is not only wicked to do harm with a drug but often unethical to omit to administer it.

In this situation, the role of regulatory authorities is as unenviable as the position of the manufacturers. The regulatory authorities exist to protect the public. The losses incurred by delaying or banning new agents are neither obvious nor, as cold statistics, heart-rending. Occasionally, however, they have been measured. Delay in the introduction of the anticonvulsant drug sodium valproate on to the market in the United States has been estimated to have subjected American patients to approximately one million unnecessary seizures a year at a cost of approximately \$200 million a year⁷⁰. Delay in the introduction of suitable anti-arrhythmic agents has been estimated to cost 10,000 deaths a year in the United States⁷¹.

Other substantial delays in introducing new drugs into the United States as compared with the United Kingdom have been carefully documented: 7 years for the first β -blocking agent for hypertension, 5 and 31/2 years respectively for cromoglycate and inhaled beclomethasone for asthma, 5 years for cotrimoxazole for urinary infections⁷⁰. It is difficult to estimate in every instance how many episodes of illness would have been prevented or treated more effectively if the drugs had been available. But presumably a sufficient quantity of evidence has accumulated in each case to justify the widespread use of the drugs. Why else did the Food and Drug Administration (FDA). in the end approve their use?

Public pressures

Unhappily, the balance sheet is not calculated solely on the best estimates of benefit and risk. Public opinion and political pressure weigh heavily against any suspicion of harm and are comparatively indifferent to benefits unless they are near miraculous. Triazure (azaribine) has been described by the director of the FDA's Bureau of Drugs as "a classic example of a highly effective drug which is useful to a small number of patients with a serious disease (psoriasis) but which also carries a serious risk of harm (thromo-embolism) in a fraction of those who take it" (J. R. Crout cited in ref. 72). It was released by FDA and later withdrawn, and its licensing became the subject of a congressional enquiry. Shubin's account⁷² of the proceedings raises serious questions about the

exercise of medical judgement in a democratic society: clearly safety as a motive over-ruled any question of medical benefit.

Science does not flourish in the political arena, and the problem becomes philosophical: is it better to take risks in the hope of benefit, or to prohibit expected benefits because of an uncertain risk? Specific regulations are not as a rule unreasonable; it is the cumulation of requirements, their inconsistency between nations and their promotion of the mechanical check list as a substitute for reason and responsibility which achieves undoubted harm and uncertain good.

No manufacturer wishes to put unsafe drugs on the market, and it is natural to do what tests appear feasible to ensure safety, but the multiplication of tests of uncertain validity in no way makes them cumulatively any more reliable. It only increases the probability of rejecting useful substances on bogus grounds. It adds to the cost of developing new drugs, which is another way of saying that it diverts resources from discovery to supposed safety testing or, worse, to extensive experiments required to refute some suspicion raised on fashionable grounds but of dubious validity. And it delays the production of new remedies, during which time patients continue to suffer and die for lack of adequate therapy. One must have

sympathy for the industry and regulatory authorities in attempting the impossible tasks of giving complete protection against drug injuries, satisfying the public and defusing those who would make political or financial benefit by exploiting such hazards. But there is no excuse for any parties promoting scientifically worthless or dubious procedures.

Resources are limited, and the diversion of resources in pursuit of safety inevitably reduces the resources available for more constructive purposes. Primum non nocere is a good doctrine for medical students to assimilate, but it is going beyond common sense to refrain from using a substance which may save a hundred lives for every one person whom it harms. It is more than ten years since the words⁷³ were published "... the public and its administative watchdogs have the choice between some risks or no new drugs at all. No problems are avoided by the multiplication of unvalidated tests". But the habit persists of over-reacting to alarming information, of seeking safety without counting the cost of approaching it, of failing to preserve a balanced and scientifically acceptable appraisal of evidence of risk and of benefit. How long will it take for the experts to gain courage and for the unattractive message to be presented to and understood by the public at large?

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NEWS AND VIEWS

A new class of contraceptives?

from H.M. Fraser

In the last three years there has been considerable progress towards new contraception based on the use of analogues of luteinizing hormone-releasing hormone (LHRH). They should have an important place as contraceptive agents, for they are innocent of the side effects of steroidal methods, have no metabolic effects and are rapidly inactivated.

The characterization in 1971 of the 10 amino acid sequence of LHRH, by Schally and Guillemin, has led to the synthesis of progressively more potent antagonists and agonists (see the figure). These can inhibit reproductive functions by decreasing or increasing the LHRH stimulus to the gonadotrophic cells in the anterior pituitary gland, modulating the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Surprisingly, the agonists (originally developed to treat infertility) have the more dramatic inhibitory effects. Their effects were first noticed during toxicity trials in rats with high doses of LHRH and its agonists; given daily, they disrupted oestrous cycles, inhibited luteal function and pregnancy and, in males, caused involution of the seminal vesicles and prostate glands and impaired spermatogenesis1-3.

The inhibitory effects of low-dose agonist treatment have since been extended to monkeys4.5 and women6-9. Early attempts to stimulate ovulation in anovular women with chronic therapy led to the finding that pituitary responsiveness is decreased, not increased. This suggested that LHRH might be used to prevent ovulation in normal women, and early studies suggested at least three possible procedures. Ovulation could be inhibited by injecting a LHRH agonist daily throughout the cycle (5 µg per day)6, an inadequate luteal phase could be induced when agonist was given at mid cycle (100 µg per day)7 and luteolysis could be provoked by giving agonist for 2 days in the late luteal phase $(50 \,\mu g \text{ per day})^{8.9}$.

The search for luteolytic agents has been one of the major goals of the contraceptive developers. One obvious advantage of such agents is that a woman would have an otherwise normal cycle. The fact that a luteolysin need only be administered at the end of a cycle would reduce the risk of

toxicity and side effects and also lower cost. But luteolytic agents are often ineffectual when conception has occurred, for their effects can then be offset by the stimulatory effects of human chorionic gonadotrophin (hCG) produced by the trophoblast¹⁰.

There is, however, no question that fertility is prevented when the agonist is administered daily to prevent ovulation. In clinical trials on 30 women in Uppsala¹¹⁻¹³ and 60 women in Berlin^{14,15} using agonist as a contraceptive for 3–12 months, no pregnancies occurred and there were no problems with reversibility. Daily injections are obviously impracticable but agonist can be administered in a nasal spray, although larger quantities (400–600 µg) must then be given.

Continual administration of LHRH agonist produces considerable changes in ovarian steroid production. Because ovulation is inhibited, the progesterone normally produced by the corpus luteum is absent, but this by itself is of little consequence. Variable and unpredictable changes in the most potent female sex steroid, oestradiol-17B, are much more important. It is essential that the oestrogen does not vary outside certain limits: too little, and women may develop menopausal symptoms, too much, in the absence of progesterone, and there will be proliferation of the endometrial lining of the uterus, predisposing it to hyperplastic change.

In about one-quarter of the women studied, the agonist prevented follicular development as well as ovulation, as indicated by consistently low concentrations of oestradiol in the blood¹¹⁻¹⁵. Thus there is little stimulation of the endometrium and women are virtually amenorrhoeic. Fortunately oestrogen was still present in sufficient amounts to avoid problems of oestrogen deficiency in all but a few women.

In the remaining women, ovarian function was less than fully suppressed, follicles still developed and oestrogen rose at varying intervals of time to stimulate endometrial growth. When the oestrogen

levels fall in the absence of ovulation, endometrial bleeding occurs. In a few women, who are presumably even less suppressed, these rises in oestrogen are followed by a small rise in progesterone indicating either ovulation with an inadequate luteal phase or luteinization of an unruptured follicle^{14,15}. In these women, bleeding is more regular.

Many women would object to such alterations of their monthly menstrual patterns. Those who become amenorrhoeic may welcome the opportunity to be free of periods once contraceptive efficiency is ensured.

The safety of chronic agonist treatment hinges on its effects on the endometrium. In the Swedish12 and Berlin study14,15, and in macaque monkeys treated with agonist for more than a year16, there was a spectrum of endometrial activity ranging from inactivity (usually when blood oestrogen was low) to marked proliferation. Although a chronically proliferative endometrium is undesirable, it will be of interest to compare endometrial activity with that of lactational amenorrhoea (low oestrogen, no progesterone) in which many women used to spend much of their reproductive lives without adverse effects.

Regular menstrual bleeding may be achieved by using lower doses of agonist15 or by confining treatment to the early follicular phase²¹, to permit ovulation followed by a defective corpus luteum. This should be incompatible with implantation but there may be some risk of an occasional fertile cycle. The best approach might be to provide exogenous progesterone for a few days at the end of a period of agonist treatment, priming the endometrium and inducing menstruation when withdrawn. Preliminary results of such a regimen are encouraging¹⁷ and although supplementation with progesterone detracts from the idea of completely avoiding exogenous steroids, it is not a return to the 'pill' since no oestrogen is given and progesterone would be administered for a short time only.

The amino acid sequence of LHRH and a widely used agonist.

AGONIST pG1u - His - Trp - Ser - Tyr - G1y - Leu - Arg - Pro - G1yMR₂ $(B\psi^{T})$ $(B\psi^{T})$ $(B\psi^{T})$ $(B\psi^{T})$ $(B\psi^{T})$

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The use of LHRH agonist as a contraceptive is probably most attractive for women over 35 who do not wish to take the 'pill' because of its minor side effects. as well as the increased risk of cardiovascular problems. The agonist might also prove ideal for lactating women, who would more willingly accept the prolongation of their lactational amenorrhoea, and in whom the combined steroidal oral contraceptive is contraindicated because of its adverse effects on lactation. Agonists used as contraceptives may also, by lowering oestrogen and inducing amenorrhoea, benefit women with menorrhagia or endometriosis while the inhibition of the cyclic progesterone rise may help relieve some symptoms of pre-menstrual tension. There are other therapeutic benefits of chronic agonist treatment, such as control of precocious puberty¹⁸, and they may also be of value in treatment of breast and prostate cancer^{2,3}.

LHRH agonists also in principle permit partners to share the responsibility of fertility control. In a recent trial in men who self-administered daily injections of LHRH agonist for 6-10 weeks, sperm numbers fell markedly19 but, as with other methods that interfere with LH as well as FSH output, testosterone concentrations in the blood also declined, so the treatment was accompanied by loss of libido, impotence and even hot flushes. Thus, testosterone replacement is required and it remains to be seen how effective or practicable this would be. Another problem is that the doses which prevent ovulation in women have no significant effect on the male reproductive function, precluding the use of nasal sprays. But, more efficient methods of delivery of LHRH analogues are expected — subcutaneous implants have already proved active⁵.

The mechanism whereby LHRH agonists exert this 'paradoxical' inhibitory effect remains a puzzle. It is believed that the pituitary is normally stimulated by pulses of LHRH from the hypothalamus to release pulses of LH into the peripheral circulation, which, together with FSH, control gonadal function. On this view, the agonists, some one hundred times more potent than endogenous LHRH, over-stimulate the pituitary with the result that the gonads are exposed to excessive gonadotrophin leading to the 'down regulation' phenomenon. Repeated exposure to agonists, on the other hand, reduces the pituitary's ability to release large amounts of gonadotrophin such as occurs at the pre-ovulatory surge13,20.

This understanding has recently been

complicated by the recognition of direct gonadal actions of LHRH, involving functions which can both stimulate and inhibit steroidogenesis, and the finding of gonadal LHRH receptors.

As things are, LHRH analogues are not the answer to rapid population growth in developing countries because of the mode of administration, yet millions of women are seeking a better alternative to the 'pill'. Moreover, as Djerassi has pointed out22, while we fear the population explosion and demand better methods of contraception, the advent of the 'pill' has also led to an excessive fear of side effects, a suspicion of new developments and an environment in which pharmaceutical companies find it financially impossible to develop new contraceptives. LHRH agonists are not free from problems, but they work, and most important, to millions of women they should be more acceptable than the 'pill'. We should tackle their shortcomings because they are our brightest prospect for a new approach to contraception.

The elixir of life

from an aging correspondent

ALEX COMFORT in his recent article Gerontology in adolescence (Nature 296, 179; 1982) declared that postponing diseases of the elderly beyond the normal life span was probably the area where most advance was likely in geriatric health. Nevertheless, he expressed the hope that an overall neuroendocrine timekeeper will be identified whch may allow modulation of life span itself. It has long been a puzzle why some vertebrate species have long life spans — sulphur-crested cockatoos, for example, have been known to live for 130 years - whereas others are unlikely to survive two consecutive seasons. Moreover, long-lived poikilotherm vertebrates, such as giant tortoises and certain fish, do not stop growing with maturity, and it is these animals that show little, if any, evidence of senescence at the cellular or tissue level. New studies suggest that specific anti-senescence factors sustain the growth of these organisms.

The species which has received most attention from gerontologists is the freshwater carp, Cyprinus carpa. The carp has an ancient lineage of pisciculture - as a source of food in Roman and mediaeval Europe and as ornamental goldfish in the Orient. Their longevity is legendary and has recently been confirmed by carbondating. Dlouhý-Zivot and Ryba (Acta gerontol. (Prag.) 26, 143; 1981) analysed the ratio of ¹⁴C in the vertebrae and scales of two carp weighing over 30 kg taken from the fish pond of Gregor Mendel's former monastery near Brno; it was evident that

these same fish had been lurking in the muddy depths several years before Mendel first bred his peas only 100 m away. The European carp grows very slowly and it would appear that the long, cold winters aid its longevity. In Israel, where carp are an important source of protein, the fish grow very much faster, but attempts to keep long-lived carp as founder-breeders for commercial strains had not, until recently, been successful in the Galilean climate (Lechaim et al. Israel J. Piscicult. 12, 86; 1979).

The endocrine basis of growth and senescence in the carp has long been the focus of attention of Obispo and his colleagues in the Stoyte Institute of Life Sciences at Tarzana College, California. Boone and Obispo (Am. J. Nutr. 42, 1213; 1960) first investigated factors in the gut because of Hauberk's remarkable claim some 125 years earlier at the age of 92 (Phil. Trans. R. Soc. B103, 397, 183; 1834) that addition of raw carp viscera to the diet each Friday prolonged fitness and sexual vigour. Obispo and Maunciple (Am. J. Nutr. 58, 82; 1967) extracted a protein factor from the intestine of carp which significantly extended the life span of gnotobiotic mice. After many a summer's hard work, detailed analysis revealed that the factor is a small protein, named longevin, comprising two dissimilar subunits, a hexadecapeptide A-chain and an icosapeptide B-chain linked by disulphide bonds (Maunciple et al. J. molec, Endocr. 2; 575; 1976). The activity of longevin is

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extremely heat labile on account of the instability of the B-chain. Three years ago Obispo's group made the notable discovery (Eldestez et al. J. molec. Endocr. 5, 902; 1979) that the B-chain was synthesized by Escherichia coli in the visceral flora of the carp whereas the A-chain was released from neuroendocrine Kultschitzky-like cells in the gut wall.

At last month's meeting of the International Gerontological Society in Athens, Obispo announced that the B-chain gene had been cloned and sequenced. Furthermore, by manipulating the sequence to code for lysine in place of arginine three residues from the C-terminus, a heat-stable molecule was obtained which still forms a functional complex with the A-chain. When bacteria expressing the modified B-chain gene in a mobilizable plasmid were introduced into 18 month-old specificpathogen-free mice, prolongation of the life span was observed (Proc. natn. Acad. Sci.; in the press). Apparently the modified B-chain forms functional complexes in the mouse gut with a host hexadecpeptide homologous to that of the carp. Introduction of the B-gene into mice genetically predisposed to progeria did not result in delayed aging, suggesting that the host A-chain was deficient in these mice.

Some peculiar side effects were noted in mice colonized by bacteria producing longevin B-chain. The pelage on the body was replaced with scales as on the tail, and about 20 per cent of the mice developed multiple carcinoid tumours of the colon. These tumours release large amounts of longevin A-chain, as well as serotonin. A cDNA library has been prepared from the tumour cells and Obispo is confident that his laboratory has already identified the A-chain gene by co-transfection of the cDNA library together with the cloned B-chain gene into senescent MRC-5 cells. He is optimistic that the administration of a plasmid containing both A- and B-chain genes into the gut flora of mice will prolong life without inducing tumours, or hopefully the scaly skin. Although he has patented the B-chain clone, he admits that treatment of humans is still some years away.

The carp is unlikely to be the only source of longevin B-chain. Gerontologists are intrigued by the unusually long life spans of cold-water fishing communities as far apart as the Falkland Islanders and the Aleut Indians (Whiting et al. WHO World Nutrition Rep. No.5, 110; 1980), where raw krill is an important source of vitamins A. D and E. In northern Hokkaido, Furō and Choju (Jap. Jl Publ. Hlth 17, 646; 1975) reported exceptional vigour among the elderly fisherfolk who eat whole salted sprats. And the parish registry on Canna in the Inner Hebrides reveals several centenarians in the nineteenth century when kippers were smoked and eaten without evisceration (Gonister J. comp. Nutr. 29. 82; 1978). Long life is a fishy business indeed.

Cancer genes — processed genes — jumping genes

from Peter Newmark

New data, some of it just published and some presented at a meeting* four weeks ago, illustrate the growing convergence of opinion on the activation of certain cellular genes (transforming genes) as the molecular basis of cancer. Whilst there is undoubtedly a danger of oversimplifying and generalizing from these studies, there is no doubt that they deserve attention, not least because of speculation that the genetic changes associated with cancer might be better understood in the light of our dramatically changed notions of the stability of the eukaryotic genome.

A variety of evidence strongly supports the view that, within the human genome, there are genes whose activation from dormancy or, at most, low-level expression is associated with the conversion of a normal cell into a tumour cell. Part of the evidence is the isolation, from human cells, of DNA sequences and RNA transcripts which cross-react with the transforming genes of tumour viruses of animals. But the most dramatic evidence of recent times is that it is possible, occasionally, to extract from human tumour cells, DNA that will transform cultured mouse fibroblasts into cells which, like tumour cells, form foci in culture and can induce tumours in mice. This DNA is said, therefore, to consist of, or to contain, a transforming gene. (It should not be overlooked, however, that these fibroblasts, NIH 3T3 cells, are not normal in all respects. Like tumour cells they are immortal in culture and they tend spontaneously to form foci in culture even before transfection with DNA from a tumour cell. What is more, they are the only type of cell that can reliably be transformed by human DNA.)

In Houston, M. Wigler (Cold Spring Harbor) outlined his group's progress (much of which appears on p.404 of this issue) in isolating and characterizing one transforming gene and R. Weinberg (Massachusetts Institute of Technology) described parallel studies with what is probably the identical gene from a different tumour. Each group started with a different human bladder carcinoma cell line and ended up with a very similar cloned fragment of DNA that retained the ability to transform NIH 3T3 cells. And both groups have used this fragment to detect similar sequences in the DNA of normal human tissues, implying that the transforming genes of tumours are neither the result of a major modification of normal genes (for example by rearrangement) nor the result of genetic import (for example, by a tumour virus).

Further support for the lack of major modification comes from Weinberg's failure to detect any differences in the way eight restriction enzymes cut the transforming gene and its normal counterpart. Wigler, however, does find some small differences but there are as many between different tumour cell lines and between different normal human placentas as there are between tumours and normal tissues. This extensive polymorphism, Wigler speculates, is due to the presence of a variable number of copies of a short sequence of DNA in the environs of the transforming gene.

It is generally assumed that transforming genes are transcriptionally inactive in normal tissue and that cell transformation is a consequence of their activation, by whatever means. Not surprisingly therefore, Wigler finds transcription of the bladder transforming gene in the cell line from which it can be transferred to NIH 3T3 cells but not in normal tissue. More surprising was his claim in Houston that transcription of the bladder gene could be detected in every tumour cell line examined, including some which have a transforming gene different from that of the bladder cell line. Both Wigler and Weinberg have extracted at least three distinct transforming genes from human cell lines derived from different tissues but have found no definite correlation between the tissue and gene types (in addition to the bladder carcinoma gene, Wigler has also cloned the distinct transforming gene of a lung carcinoma cell line).

What is the basis of the transcriptional activation of the transforming genes? One rather unlikely possibility is that it is the result of amplification of the gene. There is evidence of amplification of mammalian transforming genes on an evolutionary time scale (Chattopadhyay et al. Nature 296, 361; 1982) but on a developmental time scale there are few examples of amplification (the most recent of which is the transient amplification of chicken actin genes during myogenesis - see W.E. Zimmer and R.J. Schwartz in Gene Amplification ed. Schimke, R.T.; Cold Spring Harbor Laboratory, New York; 1982). Both Weinberg and Wigler have explored the possibility of amplification in a panel of human carcinoma cell lines with their transforming gene probes. Weinberg finds no more than a twofold difference and Wigler at most a fivefold difference between different cells with his bladder gene probe and none with his lung gene

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^{*&}quot;Perspectives on genes and the molecular biology of cancer"; the 35th Annual Symposium of Fundamental Cancer Research, held at Houston, Texas, on 2-5 March 1982.

probe. The evidence for gene amplification as a basis of transcription is therefore weak, particularly since even apparent amplification may in fact be the result of the presence of pseudogenes or of abnormal numbers of chromosomes.

It is much more likely that transcriptional amplification is the result of switching the transforming gene on (or up) by, for example, a mutation or insertion in a site that regulates transcription. In theory that could be revealed in a matter of months when the sequences of the transforming gene from bladder tumour cells and normal cells will be available for comparison but it may be difficult immediately to prove that any change of sequence is responsible for increased transcription.

Such is the situation in bursal lymphomas of chickens. There is good evidence that these are caused by the integration of avian leukosis virus (which does not carry a transforming gene) into the host genome in such a way that a cellular transforming gene comes under the influence of a viral promoter. The problem arises from the fact that the inserted promoter need not be in the expected position — just upstream of the transforming gene — but may also be downstream or even in the 'wrong' orientation (Nature 295, 209; 1982). By way of indicating that this well documented system is not unique, Harold Varmus (University of California, San Francisco) presented evidence of the localization of murine mammary tumour virus sequences within the same 6 kilobase fragment of the genome of 5 out of 35 mammary tumours caused by the virus. This localization is reminiscent of, if less frequent than, that of avian leukosis virus sequences in bursal lymphomas. So far, however, Varmus has no evidence that the 6 kilobase site contains a transforming gene or produces any transcripts.

The cellular transforming gene which is activated by avian leukosis virus is one of a class of such genes that are defined by their relationship to the transforming genes carried by many acute tumour retroviruses. (So far there is no evidence, but some expectation, that these cellular transforming genes, and those exemplified by Wigler and Weintraub, are of the same class.) It is assumed that viral transforming genes are derived from the mRNAs of cellular transforming genes. This assumption fits with the fact that both the viral genes and mRNAs lack the introns of the cellular genes. Howard Temin (University of Wisconsin-Madison) described such a case in which the rel oncogene of reticuloendothelial virus (strain T) has clearly been derived from the mRNA of a cellular oncogene that is found in birds (but not mammals). The cellular gene occupies a very big stretch of DNA because of its introns and in the turkey, the gene is highly polymorphic, perhaps because of deletion/insertion events in the introns.

The discovery of what Philip Leder (NIH and Harvard Medical School)

dubbed 'processed genes' strongly suggests that transforming genes are not the only cellular genes that can be re-integrated into chromosomal DNA. Like viral transforming genes, processed genes have a sequence closely resembling the coding sequence of a cellular gene. What was probably the best case of a processed gene, that of α -globin (Lueders et al. Nature 295. 426; 1982), has now been superseded by the case of human immunoglobulin λ light chain (Hollis et al. Nature 296, 321; 1982). This processed gene terminates in a long sequence of adenosines, like the 'poly(A)' tail of the mRNA from which it must surely have been derived by reverse transcription and integration. (Note, however, that the mRNA in question appears to have been derived from an incomplete, abnormal transcript.)

But the most striking processed gene will be that of human β -tubulin (Wilde et al. Nature; in the press). It appears to have been derived from a complete transcript. It lacks introns and has a poly(A) tail. And, it is bounded by a directly repeated 11 base pair sequence. This reminds one of the sequences that flank all transposed genes and make it yet more likely that the gene was integrated as supposed.

Another hallmark of a processed gene is

that it can be located far from the parental gene, even on a different chromosome. M. Birnsteil (University of Zurich) raised the possibility that a gene could pass not just between chromosomes but between species. In analysing the histone genes of various species of sea urchin, his group has come to the startling conclusion that within the last million years, a cluster of histone genes jumped from one species to a distantly related species with which it lives in the Atlantic Ocean. How else is one to explain the fact that there is little difference between the third base positions of one histone gene cluster of the two species whereas the difference between other clusters in these and other species is always reasonably great and consistent with a fixed rate of change with evolutionary time (M. Busslinger et al. The EMBO J. 1, 27; 1982). Was this also a virally mediated jump?

In several ways, therefore, loose but tantalizing threads hold together the genetic mechanisms of cancer and evolution. The links may be illusory but even if they vanish with time, it is more than likely that others will be forged as molecular biologists continue, in Leder's modest description, "to stumble" across new varieties of genomic fluidity.

Geomagnetic cores and secular effects

from D.H. Tarling

ALTHOUGH the geomagnetic field was the subject of the earliest scientific treatise (Gilbert's De Magnete of 1600) it still remains the most enigmatic of all the Earth's properties. The situation is even more extraordinary when it is realized that it is also the only geophysical property whose value can be determined for past times by direct measurements of rocks at the Earth's surface. Although the lack of progress reflects, in part, the complexity of the magnetohydrodynamic equations that govern core processes, the chief problem given the number of geomagnetic models available for testing - must be seen as a lack of data.

The problem has been partially resolved in this century by the establishment of geomagnetic observatories and by the increasing number of field studies, supplemented by satellite monitoring. The present geomagnetic field is now quite well known, but the long-term changes in the field, recorded in rocks, are not—they have mainly been studied for other purposes, particularly geological and archaeomagnetic dating. A recent meeting at the Royal Society of London* was thus significant in

concentrating a variety of minds on defining magnetic data in terms of core processes and complementing seismic, geochemical and geomagnetic data with theoretical considerations.

There are, in fact, numerous observations of the geomagnetic direction, especially declination (and to a lesser extent intensity), available from observations in the 18th and 19th centuries. Thompson (University of Edinburgh) and Malin (Institute of Geological Sciences, Edinburgh) have re-examined much of these data with somewhat surprising results. It seems that the lifetime of the components of the non-dipole field (isoporic foci) decay in much shorter periods, some 500 years, than had been previously thought. The present westward drift of the field has been occurring on a world scale for most of the past 400 years and a change in the sign of the quadrupole component occurred in 1820. Studies of lake sediments (Creer, University of Edinburgh; Barton, University of Rhode Island) and archaeological materials (Kovacheva, Bulgarian Academy of Science; DuBois, University of Arizona) show clearly that intervals of both eastward

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^{*}The meeting entitled "The Earth's Core: Its Structure, Evolution and Magnetic Field" was held on 27-28 January 1982.

and westward drift have occurred. Creer found evidence for both correlations and lack of correlations in lake sediments from Europe and North America which Thompson explained as the simple growth and decay of non-dipole features stationary components arising where two non-dipole components of the same sign are interacting. The presence of both clockwise and anticlockwise rotations over archaeological periods means that the westward drift cannot be adequately explained by a persistent faster rotation of the mantle relative to the core. However, the rate of secular variation and the rate of rotation of the Earth are clearly coupled, as indicated by the 1969 'jump' in secular variation (Barraclough, Institute of Geological Sciences, Edinburgh), but the lunar tidal deceleration of the mantle of 1.78 ms per century may be accompanied by a nontidal acceleration of 0.8 ms per century (Runcorn, University of Newcastle) attributable to a continued but very slow growth of the core, as also suggested on geochemical grounds (Allegré, University of Paris).

All geomagnetic models are constrained by the need to allow polarity reversals. It is generally agreed that reversals occurred randomly during over the past 48 million years but may have occurred more systematically before that. However, it is probable earlier reversals have simply not been dated with sufficient precision. Lowrie (University of Zurich) and Channell (Lamont-Doherty Geological Observatory) presented improved biostratigraphical dating of reversals in the Mesozoic that are vital for establishing world-wide correlation scales. Unfortunately, analyses of the geomagnetic polarity frequency still require conversion of the biostratigraphical time scales into an accepted radiometric ('absolute') scale. The new radiometric decay constants for K/Ar have been designed to give a greater uniformity in radiometric ages determined by different methods (Steiger & Jager Earth planet. Sci. Lett. 36, 359; 1977), but consistent errors of several per cent can still arise in establishing the radiometric age of biostratigraphical horizons (Tarling & Mitchell Geology 4, 133; 1976). Careful analyses and assessments are therefore necessary before the polarity frequency in the early Tertiary and Mesozoic can be considered to be established.

Polarity transitions are likely to provide key diagnostic information on the processes operating in the geomagnetic dynamo but have proved very difficult to study. Thin slices of oceanic sediment cores can be used to determine more precisely the changes at specific locations along a line of longitude (Opdyke, University of Miami), but observations are mostly confined to the last geomagnetic transition and to the Northern Hemisphere (Hoffman, California Polytechnic). Sediments can, in any case, be disturbed by non-geomagnetic effects and Verosub (University of California Polytechnic).

fornia, Davis) remains rightly unconvinced about much of the available sedimentary evidence for most polarity excursions, even though some undoubtedly exists. Even records in igneous rocks may be dominated by rock magnetic factors, such as in the Laschamp-Olby excursion (Heller, University of Zurich), and studies of igneous sequences, without adequate radiometric control, can also be misleading. It is now clear that the 'dipole window' in the Pacific did not exist over the past few million years or so, but is simply an artefact of the very rapid eruption rates in Hawaii (McWilliams, University of Stanford), although the corresponding palaeomagnetic poles continue to be slightly 'far-sided' and right-handed, as in Iceland, posing problems in any interpretation of the average geomagnetic field (Harrison, University of Miami).

The geomagnetic dynamo does not require a large driving energy and sufficent could be provided by the solidification of the inner core. The outer core is probably enriched in carbon, sulphur and oxygen (Ahrens, Caltech), so it is likely that further energy is provided by the incorporation of some radiogenic heat-producing elements, such as K, Th and possibly U. The core has generally been neglected in considering the distribution of lead and anomalies found in rocks derived from the mantle have often been interpreted as showing that mantle convection could not take place. Allegré considered that the core could, in fact, be a major factor in the continuing evolution of the core and mantle.

The evidence for actual motions in the core was somewhat controversial, with LeMouel (University of Paris) considering that there are clear signs, in geomagnetic measurements, for such motions, while these were not seen by Gubbins (University of Cambridge). The most provocative point concerned whether or not there are 'bumps' on the core-mantle interface. These are now more closely defined by the seismic evidence (Bolt, University of California, Berkeley), particularly when observed signals are compared in both amplitude and shape with theoretical seismograms. The 'scattering structures' appear confined very closely to the coremantle boundary with lengths some 10-30 km and heights of a few 100 m - if they are topographical 'bumps' (Haddon, Bureau of Mineral Resources, Ottawa). However, they could equally well mark zones of temperature or physical differences rather than topographical boundaries. Either explanation may, of course, also be linked to the convective pattern in the mantle (Hide, Meteorological Office, Bracknell). The presence of numerous bumps may cause an increased amplitude in secular variation and also an increased probability of reversals of polarity, and quieter magnetic periods, on a geological time scale, may be associated with fewer bumps. This demonstrates, yet again, the need for closer examination of the palaeomagnetic records to see whether there really is any correlation between the magnitudes of secular variation and the frequency of polarity transitions.

A galaxy for quasar 3C48

from R.F. Carswell

THE superb observations by Boroson and Oke reported in this issue of *Nature* (see p.397) reveal that the faint nebulosity to the north and south of the quasar 3C48 is dominated by starlight and that the stars have a redshift close to that determined from the broad emission lines in the quasar spectrum. This provides the first direct evidence that a quasar is associated with a galaxy and lies at a distance corresponding to its redshift, adding to the weight of evidence against non-cosmological quasar redshifts.

Most quasars are so far away that there is little hope of detecting galaxies which might be associated with them, but for a number of low-redshift examples, faint surrounding nebulosity has been found. The difficulty has then been to establish that this extended emission arises from starlight and not from a large gaseous envelope which radiates only in emission

R.F. Carswell is in the Institute of Astronomy, University of Cambridge, Madingley Road, Cambridge CB3 0HA. lines. A number of attempts have been made to do this, by obtaining spectra of the nebulosity to search for the stellar absorption lines typical of galaxies, but until now the results have been somewhat unconvincing.

While Boroson and Oke's work confirms a few widely held beliefs, the results also contain surprises. The first, and most obvious, is the discovery that the stars in the 3C48 galaxy are not quite like those in a normal galaxy such as our own. They appear to be bluer, and younger, than usual, similar to those in galaxies where recent bursts of star formation are thought to have occurred. Estimates of age are difficult, but the brightest part of the stellar population in 3C48 is probably 100 million to a billion years old. If the stars have formed recently we would expect the system to be rich in gas, and certainly the material presented by Boroson and Oke shows, through emission lines in the spectra of the nebulosity, that there is still much gas present. The galaxy could thus be a gas-rich spiral rather than an elliptical which has little interstellar material, though given the distance of 3C48 it will be very difficult to confirm the presence of spiral structure.

It is also tempting to suggest that those quasars with strong emission lines are formed in spiral (that is, gas-rich) galaxies. In all cases, only very small amounts of gas are needed very close to the quasar to explain the line strengths seen, but then only a small amount of the available gas will be in the immediate quasar neighbourhood. BL Lac objects, which are really quasars with no emission lines and therefore probably little gas in their neighbourhood, could similarly be in the centres of elliptical galaxies (which have a low gas abundance). The late-type stellar spectrum recently found for the nebulosity around BL Lac is itself suggestive in this regard. If young stars do generally dominate the quasar stellar spectrum, this incidentally helps to

explain why previous searches for starlight have failed. Most of these have looked primarily for the usual strong Ca II feature typical of a later population, which is weak or absent in the 3C48 spectrum. But much work remains to be done before this can be established; one object, however important, is hardly a statistical sample!

Another interesting, and more puzzling, result is that the redshift of the quasar determined from the galaxy starlight does not agree very well with that determined from the sharper emission lines in the quasar spectrum. While it is not unusual for the different broad emission lines to give rather different redshifts, the sharp lines are believed to arise in a more extended region and so to give a better redshift estimate. Here the sharp lines differ from the galaxy redshift by about 400 km s⁻¹ while the broad hydrogen lines agree very well. It is possible that a combination

of velocity flow and obscuration of the clouds shifts the sharp lines significantly, but the required shift is so large (comparable with the line widths) that it is rather difficult to explain simply.

Another surprise is simply one of observational detail. The spectra Boroson and Oke obtained for two regions of the nebulosity around 3C48 took a total of only two hours of telescope time, while others in the past have unsuccessfully devoted much longer periods of time to similar projects. A combination of an efficient spectrograph and a modern charge-coupled device as a detector has allowed them to obtain useful spectra of faint objects quickly. With such a system available one hopes, and expects, that similarly exciting discoveries will continue to be made at Palomar, and that Boroson and Oke will be able to follow up their observations by examining the nebulosity around other quasars.



100 years ago

THE WINGS OF PTERODACTYLES

The first Pterosaurians discovered were recognised as flying animals, but were thought to be bats. As soon as their general structure became known, they were classed with the reptiles, although it was considered possible that their power of flight was due to feathers. Later their bones were mistaken for those of birds by various experienced anatomists, and others regarded them as sharing important characters with that group. Some anatomists, however, believed that the fore-limbs of pterodactyles were used for swimming rather than for flight, and this view has found supporters within the present decade. A single

fortunate discovery, made a few years since, has done much to settle the question as to the wings of Pterodactyles, as well as their mode of flight, and it is the aim of the present article to place on record some of the more important facts thus brought to light.

The specimen to be described was found in 1873, near Eichstädt, Bavaria, in the same lithographic slates that have yielded Archoeopteryx, Compsognathus, and so many other Jurassic fossils known to fame. This specimen, which represents a new species of the genus Rhamphorhynchus, is in a remarkable state of preservation. The bones of the skeleton are nearly all in position, and those of both wings show very perfect impressions of volant membranes still attached to them. Moreover, the extremity of the long tail supported a separate vertical membrane, which was evidently used as a rudder in flight. These peculiar features are well shown in Fig. 1, which represents the fossil one-fourth the natural size.

A careful examination of this fossil shows that the patagium of the wings was a thin smooth membrane, very similar to that of modern bats. As the wings were partially folded at the time of entombment, the volant membranes were naturally contracted into folds and the surface was also marked by delicate striae. At first sight, these striae might



Fig.3 Restoration of Rhamphorhynchus phyllurus.

readily be mistaken for a thin coating of hair, but on closer investigation they are seen to be minute wrinkles in the surface of the membranes, the under-side of which is exposed. The wing membranes appear to have been attached in front along the entire length of the arm, and out to the end of the elongated wing finger. From this point the outer margin curved inward and backward, to the hind foot.

The membrane evidently extended from the hind foot to near the base of the tail, but the exact outline of this portion cannot at present be determined. It was probably not far from the position assigned it in the restoration attempted in the cut given below, Fig. 3. The attachment of the inner margin of the membrane to the body was doubtless similar to that seen in bats and flying squirrels.

In front of the arm there was likewise a fold of the skin extending probably from near the shoulder to the wrist, as indicated in Fig. 3.

The present species appears to be most nearly related to *Rhamphorhynchus Gemmingi*, von Meyer, from the same geological horizon, and near the same locality. That it is quite distinct, however, is shown, aside from the difference in size, by the complete ankylosis of the scapula and coracoid, and by the fifth digit of the hind foot being well developed, and having three phalanges. In the name *Rhamphorhynchus phyllurus*, here proposed for the species, the latter designation refers to the leaf-shaped caudal appendage, which appears to be one of its most characteristic features.

For the long delay in the description of this important European specimen, the writer can only plead *l'embarras des richesses* nearer home.

O.C. Marsh

Yale College, New Haven, March 14.

From Nature 25, 531, 6 April 1882.



Fig. 1 Rhamphorhynchus phyllurus.

ARTICLES

Detection of the underlying galaxy in the QSO 3C48

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Spectra have been obtained of the faint nebulosity north and south of the centre of the QSO 3C48. In addition to the emission lines previously known, a continuum dominated by hot stars is seen at both positions. This suggests that the host galaxy is a spiral and may explain why searches for features indicative of an old stellar population in QSOs have been unsuccessful. The redshift of the underlying galaxy is the same as that of the permitted lines in the QSO but differs from the redshift of the forbidden lines in the QSO by 500 km s⁻¹.

THE QSO 3C48 has long been known to be associated with wisps of nebulosity. These extend predominantly north and south from the central object and have been well illustrated by Sandage and Miller¹. Although it has been argued that QSOs occur in the nuclei of galaxies and that the underlying galaxy should be visible in some cases², this identification of the nebulosity in 3C48 has been questioned for two reasons. First, its radial extent and total luminosity are uncharacteristically large for a normal galaxy³. Second, the spectrum of the fuzz has been obtained by Wampler et al.⁴ who found no evidence for starlight. They did detect emission lines from [O II], [O III], and [Ne III] at a slightly larger redshift (0.370) than that of the same lines in the QSO (0.368). Subsequently, Bergeron⁵ modelled the spectrum of the nebulosity with an extended gaseous disk, ionized by the QSO.

Observations

We observed 3C48 and the surrounding nebulosity on two nights, 4 November and 16 December 1981 with the Hale 5-m telescope. Instrumentation consisted of the double spectrograph with an RCA 320×512 CCD on the blue camera and a Texas Instruments 800×800 CCD on the red camera. The blue camera exposures were of poor quality and will not be discussed. The grating was set to give coverage from $\lambda\,5,000$ to $\lambda\,10,000$ with the red camera, yielding about 6 Å per pixel. A slit 2 arc s wide, orientated in the east—west direction was used in all exposures.

On 4 November 1981, we obtained a 500-s exposure of the nucleus and a 3,000-s exposure centred 2 arcs north of the nucleus of 3C48 in 2 arcs seeing. On 16 December 1981, we obtained an additional 500-s exposure of the nucleus and a 3,000-s exposure with the slit centred 2 arcs south of the nucleus. The seeing on the second night was 1 arc s. Comparison lamp spectra were obtained on each night immediately after each pair of exposures of 3C48.

The data were reduced by first correcting the twodimensional image for bias level and small scale sensitivity variations. The sky spectrum was then determined and subtracted and a 4 arcs wide swathe was extracted to create a one-dimensional spectrum. The comparison lamp spectra were similarly extracted and fit with a cubic wavelength polynomial. Residual errors from these fits were <0.20 Å for each of nine comparison lines. Finally, the nuclear and nebulosity spectra were corrected approximately to absolute fluxes using observations of spectrophotometric standards obtained on each night. All observations were made within 15° of the zenith so atmospheric refraction effects are negligible.

Figure 1 shows the nuclear spectrum obtained on the first night and the spectra of the two regions in the nebulosity. The strongest lines in the nucleus have been labelled. The spectrum of the north nebulosity is contaminated by scattered light from the nucleus. This can be seen from the broad H β and H α and from the Fe II complexes. Attempts to remove this contamination by subtracting a fraction of the nuclear spectrum from the north nebulosity spectrum showed that this technique is incapable of removing the scattered light at all wavelengths. Because many of the strong features expected in the integrated light from stellar populations are between $\lambda 3,900$ and $\lambda 6,000$ we limited our efforts to this spectral region. We found that the subtraction of 1.4% of the nuclear spectrum from the north nebulosity removes all traces of the broad nuclear lines except for $H\alpha$. This corrected north-nebulosity spectrum and the uncorrected south-nebulosity spectrum, which appears essentially free of scattered light from the nucleus, are shown in Fig. 2. For ease of identification of features, the spectra are plotted against rest wavelength, assuming a redshift of 0.370. We have not plotted the spectral region longward of $\lambda 5,500$ where the data become quite noisy and the residual contamination by the nuclear scattered light may be considerable.

Results

Figure 2 shows that in addition to the emission lines seen by Wampler et al.⁴ a continuum with absorption features is present in both north and south spectra. These absorption features consist primarily of the higher Balmer series lines and the Ca II K line at $\lambda 3,933$. H β appears to be completely filled in by emission and H γ partially so. None of the absorption features indicative of late type stars, particularly the G band ($\lambda 4,300$), Mg b ($\lambda 5,175$), or the Na D lines ($\lambda 5,890$), are seen. The [O I]

Table 1 Measured redshifts of 3C48						
Line	Nuc Run 1	leus Run 2	Nebulosity north	Nebulosity south		
[O n]\ 3,727	0.3688		0.3716			
Ne m \(\lambda\) 3.869	0.3686	0.3686				
Omla4,959	0.3680	0.3685	0.3707	0.3708		
[O m]λ 5,007	0.3683	0.3688	0 3706	0.3700		
Hγ emitted	0.3702	0.3705				
Hβ emitted	0.3695	0.3698				
Ca II \(\alpha \),933 absorbed			0.3713	0 3697		
He absorbed			0.3704	0.3701		
$H\delta$ absorbed			0.3721	0.3707		
Means*						
Forbidden lines	0.3685	±0.0001	0.3710	0.3704		
Permitted lines	0.3700=	±0.0002	0.3713	0.3702		
All lines			0.3711	0.3703		
			± 0.0003	±0.0002		

^{*} Means for nucleus are average of both runs.

 $\lambda 6,300$ line, which is outside the spectral limits of Fig. 2 is also present in the nebulosity. It can be seen in Fig. 1 to be much stronger in the north-nebulosity spectrum than in the nuclear spectrum. The nebulosity apparently shows the spectrum of a hot stellar population, with a mean spectral type of about A7 as judged from the relative strengths of He and Ca II K and from the strengths of the higher Balmer lines. The continuum slope is also indicative of blue stars. The approximate B-V colours are 0.35 for the north nebulosity and 0.22 for the south nebulosity. These estimates are probably only accurate to ± 0.1 mag.

It was pointed out by Wampler et al.⁴ that the forbidden lines in the fuzz have a slightly higher redshift than those in the nucleus. Similarly, Thuan et al.⁶ found a discrepancy between the redshifts of permitted and forbidden lines in the nucleus. Therefore, we have measured the redshifts of all reasonably strong and sharp lines in the nucleus and in the nebulosity: these are listed in Table 1. All the lines in each nebulosity spectrum have the same redshift. Both of these redshifts are consistent with the redshift of the permitted lines in the nucleus. The forbidden lines in the nucleus, however, have a velocity which is, in the rest frame of the QSO $(\Delta z/(1+z))$, $330\pm78\,\mathrm{km\,s^{-1}}$ less than that of the permitted lines and $481\pm$

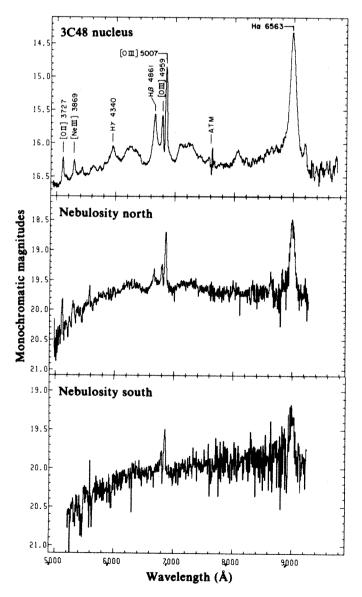


Fig. 1 Spectra of the nucleus of 3C48 and two regions in the nebulosity. The strongest spectral features in the nucleus are labelled. The feature ATM is due to the atmospheric A band.

 $106~\rm km~s^{-1}$ less than the mean of the redshifts of the two regions in the fuzz. The uncertainties quoted are derived from the mean errors in averaging the redshift determinations from the various lines.

Discussion

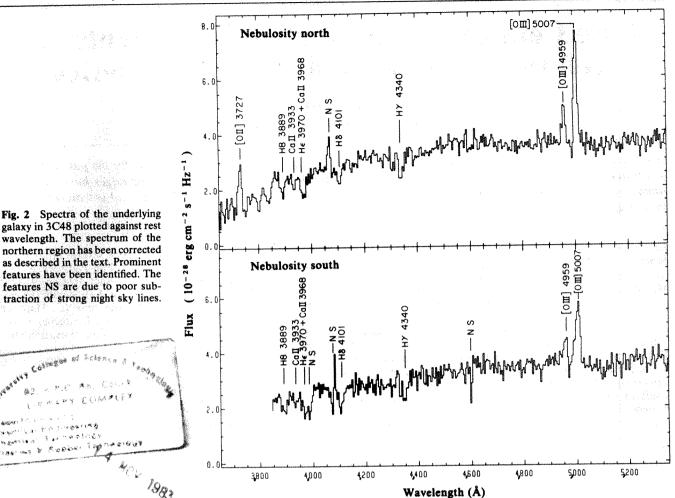
Several recent studies have used imaging techniques to produce evidence for extended structure in low redshift OSOs7 identification of this fuzz with galaxies, however, rests on circumstantial evidence. The few objects in which the nebulosity has been observed spectroscopically have shown only emission lines, that is, no evidence of a continuum or absorption features indicative of stars^{4,9-11}. The one possible exception to this is 3C206; the fuzz around it shows a red continuum with Ca II H and K absorption¹². However, other absorption lines expected to be strong are absent. Also, attempts to show the connection with galaxies by observing the highly diluted stellar features in the nuclei of QSOs have been unsuccessful¹³, unlike similar studies of N galaxies and BL Lac objects^{14,15}. Thus, the discovery of unambiguous stellar absorption features in the fuzz around 3C48, a fairly luminous QSO ($M_V = -25.3$ for $q_0 = 1/2$) is a crucial link in relating QSOs to active galactic nuclei and to normal galaxies.

The immediate goal of identifying the fuzz around QSOs is to ascertain the properties of the regions in which QSOs form. In that regard the early spectral type of the underlying galaxy is unexpected. In general, of course, blue colours and recent star formation are indicative of spiral galaxies as opposed to ellipticals. However, there are elliptical galaxies with young stars, NGC1275, for example 16. Also, the average B-V colour of the fuzz, 0.28 is bluer than a typical galaxy of even the latest type 17. The mean B-V colour for Sc galaxies is 0.51 (ref. 17), more than 0.2 mag redder than is observed in the fuzz of 3C48. The presence of gas required to explain the emission lines also argues against the host galaxy being an elliptical as these usually have little or no detectable gas 18.

The surprisingly blue spectrum of this nebulosity raises the question of whether this is a common phenomenon in galaxies in which QSOs reside, perhaps because shocks emanating from the central source trigger global bursts of star formation. It is clear that the spectrum is really dominated by the hot stars as there is no evidence for a cooler population even at Mg I b $\lambda 5,175$ or Na D $\lambda 5,890$ where the old population should contribute a larger percentage of the integrated light than in the blue. The single burst models of stellar populations calculated by Larson and Tinsley¹⁹ have B-V colours in the observed range after $1-2\times10^8$ yr. However, the mean spectral type of A7 at $\lambda 4,000$ argues for an age more likely 10^9 yr. This discrepancy, if real, may indicate a very flat initial mass function which would produce a slower reddening in B-V with age¹⁹.

Another aspect of the extreme youth of the stellar population is the total luminosity of the underlying galaxy. It has been suggested in previous work that the nebulosity is too luminous to be a galaxy3. We estimate that the total apparent magnitude is 18.5 or brighter at $\lambda 5,500$ in the rest frame of the galaxy; this corresponds to an absolute visual magnitude of -23.0 $(H_0 = 50, q_0 = 1/2)$. While this is particularly luminous for a normal spiral or elliptical galaxy, the blue colour corresponds to a population with a mass-to-light ratio, $M/L_{\rm V}$, at least a factor of 10 smaller than those typical of older populations¹⁹. Thus, this galaxy could have become as much as 3 mag brighter because of the presumed burst of star formation. If this is not unusual, that is, if QSOs frequently occur in gas rich galaxies in which they trigger global episodes of star formation, the technique of searching for weak stellar absorption features in the nuclei of QSOs may be futile.

Wampler et al.⁴ observed the north nebulosity at twice the distance from the nucleus of the observations described here. They found no evidence for the stellar continuum at the more distant position, but did find emission lines from forbidden



transitions of OII, OIII, and NeIII. They and later Bergeron⁵ argued that the emitting gas was ionized by the QSO. Bergeron's detailed analysis had trouble explaining the absence of $H\beta$ emission which seemed to rule out heating by a Lyman continuum source. In our spectra, it is clear that the apparent absence of H β emission is due to the presence of H β absorption in the underlying continuum and also that the intrinsic strength of the $H\beta$ emission is approximately the same as the [O III] \$4,959 line. In support of the idea that the ionization is due to a power law continuum, we note the strong presence of three levels of ionization of oxygen; we have not, however, performed a detailed analysis of the line strengths.

One of the peculiar aspects of the fuzz is the redshift discrepancy. Table 1 shows that the forbidden lines in the QSO are 300-500 km s⁻¹ blueshifted from both the permitted lines in the QSO and all of the lines in the underlying galaxy. Presumably, then, it is these forbidden lines in the QSO which differ from the systemic redshift. This phenomenon has been seen in Seyfert I systems and QSOs previously^{6,20} but it has never been clear which lines, the forbidden or the permitted, represent the systemic velocity. Explanations proposed for this discrepancy include models with infalling or outflowing gas in which one side of the region is preferentially seen because of dust mixed with the gas or light travel time arguments20-22

Conclusion

We have detected stellar absorption lines in the nebulosity around the QSO 3C48. The integrated light from this population has both a B-V colour and absorption features indicative of middle to late A stars, suggesting that the underlying galaxy is a spiral in which a massive star formation burst was triggered at least 10^8 years ago. The [O II] $\lambda 3,727$ and [O III] $\lambda 4,959$ and $\lambda 5,007$ emission lines seen by Wampler et al. as well as [O I] $\lambda 6,300$ are also present. The underlying galaxy must have an absolute magnitude of -23, or brighter but without the recent burst of star formation it would be at least 2 mag fainter. The redshifts of both absorption and emission lines in the fuzz are inconsistent with the forbidden lines in the nucleus but, within measurement errors, are the same as that of the permitted lines. This indicates that the nuclear forbidden lines are blueshifted 300-500 km s⁻¹ with respect to the systemic velocity.

This work was supported by NASA through grant NGC-05-002-134.

Received 26 January; accepted 17 February 1982.

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Late Cenozoic uplift of stable continents in a reference frame fixed to South America

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A reference frame fixed to South America may approximate to an 'absolute' reference frame for the past 100 Myr. In this frame, otherwise stable continents that have moved onto the former positions of oceanic ridges have experienced rapid plateau uplift, probably caused by phase changes in the continental mantle, and occasional volcanism with time lags of up to 70 Myr.

ALTHOUGH plate tectonics accounts quantitatively for many geological phenomena, it does not explain-even qualitatively-large-scale continental uplift in otherwise stable areas. Migration of continents over 'hotspots' may account for uplift of continental areas that are as much as 1,000 km across¹, but is not applicable to areas such as southern Africa where hotspots of the appropriate dimensions seem to be absent. Yet heating is the only process that could bring about the necessary uplift. Heating can cause expansion as in the oceans2, or it can induce a phase change^{3,4}, which will also be accompanied by thermal expansion. Quantitative continental uplift models attribute uplift to some form of thermal anomaly in the continental asthenosphere⁵. Given a thermal anomaly, its passage through the continental lithosphere to the surface may cause effects that correspond closely to field observations, but possible causes of the anomaly-chemical and/or thermal mantle 'plumes', convection or shear heating—cannot yet be discriminated from one

The hypothesis advanced here is that a small amount of heat has been put into the base of the continental lithosphere where the past positions of ocean ridges younger than ~100 Myr, relative to South America, now lie under continents. I demonstrate that two such areas—'high Africa', and south-east Australia—have experienced strong plateau uplift some 50–60 Myr later, whereas adjacent continental areas, which lie just beyond these former ridge positions, have not. Moreover, in south-east Australia the pattern of Cenozoic volcanism lags behind in a remarkably systematic way the apparent traces of newly formed ocean floor under that continent.

Late Cenozoic uplift of Afro-Arabia

Africa is the continental part of a plate that includes much of the south-east Atlantic Ocean and the south-east Indian Ocean. Plate margins lie close to Africa only on its northern and north-east margin. A nascent plate margin may run along part of the African rift system. The African continent has undergone several uplift cycles⁶, the most recent of which is the Plio-Pleistocene uplift ('cymatogeny') that has created the present division of Africa into 'high Africa' and 'low Africa' (Fig. 1a). Significant uplift may have started as early as Miocene time (C. Hartnady, personal communication). Before the late Cenozoic, most of Africa was a continent of low relief, with an "elevation above sea level rarely more than 600 m". The Plio-Pleistocene cycle of uplift raised most of high Africa by ~1,200-1,500 m (ref. 6).

As might be expected, high Africa is associated with negative Bouguer gravity anomalies^{7.8}, whose source is attributed to variations in the level of a boundary between a deeper low density layer and an overlying high density layer in the upper mantle. This boundary can be regarded as the base of the lithosphere.

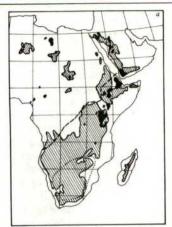
The lithosphere can also be defined by combining Bouguer anomalies with teleseismic delay times⁸, or by heat flow models⁵. Though differing in detail, all models agree that high Africa is underlain by generally thinner lithosphere than is low Africa. Thickness estimates vary considerably for low Africa: from about 250 (ref. 5), 225 (ref. 8) to 100 (ref. 7) km, with similar variations for high Africa. Recent work suggests that 'normal' continental lithosphere may be no thicker than oceanic lithosphere by that is 100 km. As a compromise, I take the lithosphere under low Africa today to be ~150 km thick, its inferred Cretaceous thickness under high Africa¹⁰.

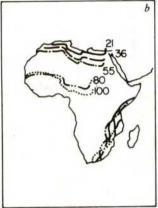
Uplift of the continent due to the physical expansion of the lithosphere should also affect the adjacent ocean floor because the boundary between high and low Africa reaches the coast. Inspection of the ocean floor bathymetry¹¹ reveals no substantial topographic anomaly across the Atlantic seaward extension of this boundary, though the effect may be negligible. Thus the topographic anomaly is largely restricted to the continent and does not appear to have its origin mainly in the thermal expansion of hot rock. This conclusion is supported by thermal calculations.

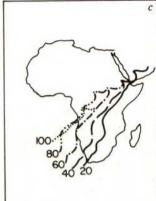
Table 1 Distance in degrees of global mean palaeomagnetic pole from geographical pole (figures not in parentheses) and distance travelled in time interval (figures in parentheses)

Continent	20	20-40	40	40-60	60	6080	80	80–100	100 Myr
Antarctica	5.7	(1.6)	4.5	(8.1)	6.1	(3.1)	3.6	(11.7)	10.2
Africa	8.2	(2.1)	9.6	(7.8)	14.2	(6.0)	20.2	(4.6)	23.8
Eurasia	6.5	(1.0)	7.2	(2.7)	8.2	(2.6)	10.3	(4.2)	11.8
North America	7.8	(1.8)	9.6	(4.3)	11.9	(5.3)	11.1	(1.0)	12.0
South America	6.0	(2.7)	5.6	(4.0)	3.3	(2.8)	6.1	(5.6)	3.2
No. of poles	89	(****)	60	,,	72	()	46	(,	52
α ₉₅	2.5		4.4		4.3		4.7		4.7

The 20 and 40 Myr data are from ref. 22; the 60, 80 and 100 Myr values use the same data base but exclude all Indian poles because of the problem of weighting poles from a rapidly moving fragment. Distances are the distance in degrees of the global mean palaeomagnetic pole calculated for the named continent from the geographical pole. The distance travelled by the pole in successive 20-Myr intervals has been calculated from successive pole positions. These can be non-zero even if the mean pole has remained at a fixed distance from the geographical pole. The global mean pole averages all known palaeomagnetic data up to 1979 from stable continental fragments on appropriate continental reconstructions the second pole for India and Australia have been omitted because these continents move substantially with respect to the poles in this time interval.







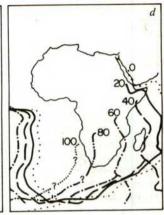


Fig. 1 a, Africa showing the distribution of Cenozoic volcanic rocks (black); the Karroo igneous rocks (within dotted line); land higher than 1,000 m (shaded); 80-Myr boundary of Africa relative to South America (dashed line). Note mutual exclusion of Karroo and Cenozoic volcanism; close similarity of northwestern edge of high Africa (essentially the 1,000-m contour) and 80 Myr boundary of Africa relative to South America. (Lambert equal area projection centred on 0° N, 15° E.) b, The past positions of Africa in the hotspot reference frame 18. There is no correlation between these positions and the boundary between high and low Africa. In b, c and d numbers are ages in Myr and cylindrical equidistant projection is used. c, Successive former positions of eastern edge of Afro-Arabia relative to South America superimposed on present-day Africa. d, Successive former positions of north-west Indian Ocean ridge under present-day Africa in past 60 Myr. The ridge positions at 80 and 100 Myr are implied by continental reconstructions, but are not known from ocean-floor anomaly data. The ridges are shown relative to a fixed South America.

For example, if it is assumed that the asthenosphere under the rift valleys is essentially at the Moho⁷, then a 150-km lithosphere under low Africa implies that much of the upper mantle under the rift valleys has been reduced in density by 26 kg m^{-3} . (Brown and Girdler⁷ adopted a thinner reference lithosphere (100 km), which requires a density contrast of 40 kg m^{-3} .) Using representative values of specific heat and thermal expansion¹², it can be shown that the heat required to bring about this density decrease by expansion alone is about $1.6 \times 10^{11} \text{ kJ m}^{-2}$ of surface area, implying an average temperature increase of $450 \, ^{\circ}\text{C}$, which is absurdly high.

Similarly improbable values are obtained from heat flow calculations. For example, the temperature increase at the base of a 150-km thick lithosphere needed to cause 1,200 m of uplift is about 1,000 °C (ref. 5), which is far too high. Alternatively, 1,200 m of uplift will take at least 100 Myr (ref. 5) if the heat flux at the base of a 150-km plate is increased by ~94 mW m⁻¹, about twice the average continental heat flow. All these thermal models require the crust under southern Africa to have partially melted, creating abundant magmatism in an area that is notably free from it. Despite their success in explaining ocean-floor subsidence², conduction uplift models cannot account for rapid plateau uplift of continents.

Phase change?

I suggest that the uplift of high Africa is most reasonably interpreted as a phase change. I assume the phase change to be analogous to the basalt/eclogite phase change and to occur at the Moho itself, taken initially at 40 km depth. The density change is from a relative density of 3.4 to 3.0. To create 1.2 km of uplift, 9 km of eclogite will have been converted to 10.2 km of 'basalt'. An analogous reaction is that of quartz eclogite to granulite.

Both reactions can be idealized, the first as (in moles)

- 3 Forsterite + 3 Diopside + 5 Plagioclase [An₆₀Ab₄₀]
 - = 3 Garnet + 5 Omphacite [=Jd₄₀Di₆₀]+2 Quartz

and the second as:

- 2 Enstatite + 2 Plagioclase + Diopside
 - = Garnet + 2 Omphacite + 2 Quartz

The enthalpy change for the quartz eclogite to granulite reaction can be estimated from thermochemical data at 10⁵ Pa (refs 13, 14) and extrapolated to high pressures. It probably lies between

 -24.7 kJ kg^{-1} at 10^9 Pa and -46.0 kJ kg^{-1} at $1.5 \times 10^9 \text{ Pa}$. The enthalpy change for eclogite to 'basalt' at atmospheric pressures is very similar to that of quartz eclogite to granulite and is assumed to have a similar range at high pressures. These values probably have an uncertainty of 8 kJ kg⁻¹. For simplicity I adopt a value of 46 kJ kg⁻¹. The total heat required to convert 9 km of 'eclogite' to 10.2 km of basalt is 1.4×104 kJ m⁻² of crosssection. Thus the thermal energy needed for uplift due to a phase change is 100 times more effective than the energy needed for thermal uplift. The heat needed for a phase change uplift is equivalent to increasing the average temperature of the lithosphere by 4 °C accompanied by 11 m of uplift. Such a small uplift cannot yet be detected in the residual depth anomalies of the adjacent ocean-floor. However, unlike thermal expansion, the active position of a phase change will be limited to a relatively narrow zone, rather than affecting all rocks, whatever their nature.

The numerous Mesozoic eclogite-bearing kimberlite pipes of southern Africa¹⁵ demonstrate the presence of eclogite in Mesozoic time in the upper mantle of southern Africa. Those eclogites containing diamonds come from much deeper levels, but others probably lay in the depth range of a possible phase change. The grain textures in some of the kimberlite inclusions show that during the Cretaceous there was a marked textural discontinuity at ~150 km depth where the temperature is estimated to have been ~1,200 °C (ref. 10). This textural discontinuity implies a rapid stress drop at this level and can be taken as marking the Cretaceous lithosphere–asthenosphere boundary. Thus 70 Myr or more ago, eclogite was present in the upper mantle of southern Africa, and the lithosphere was

Table 2 Instantaneous absolute rotations of the American plate for three different models¹⁹

Model parameters	Lat.	Long.	Rotation rate (10 ⁻⁷ deg yr ⁻¹)
B4: continents have 3 times more drag than oceans	-62.0	-108.2	1.65
C4: drag under plates; slab pull at oceanic and continental	-46.5	-89.5	1.72
subduction zones D1: maximum pull by slabs and plate drag	-86.9	-61.3	0.89

150 km thick. How much eclogite was present and where it was distributed are not known, but similar kimberlite diatremes occur in the Colorado Plateau and in south-east Australia^{16,17}. In both these regions the mantle in the 40–70-km depth range might have contained up to one-third eclogite and other high pressure assemblages capable of inverting to lower density rocks^{16,17}.

The thermal time constant due to temperature changes of the continental lithosphere is ~63 Myr (ref. 9). For a phase change at the Moho, the time constant for heat originating at the base of the lithosphere is smaller. The minimum extra heat flux required for a phase change is the heat needed divided by the time available, or 4.6 mW m⁻². This heat is the minimum extra heat flux required above the value prevailing just before 10 Myr, because 10 Myr or so ago the latest phase of African uplift was just about to start. It would be less if uplift started earlier (C. Hartnady, personal communication). Clearly, a phase change overcomes the temperature problem, but it would not be expected to give such a rapid uplift pulse as is inferred from the geomorphology. Presumably, the sharpness of this uplift is caused by simultaneous thermal thinning of the lithosphere by the heat pulse as it moves upward⁵. Thus we are looking for a thermal pulse originating below Africa some time ~70 Myr ago, that probably thinned the lithosphere-because the isotherms would have been raised—and has augmented the previous heat flux at ~ 40 km by at least 4.6 mW m⁻², or about one-tenth of the average continental heat flow.

The increase in surface heat flow will be much less because the phase change itself will initially absorb much of the heat. The increase in temperature, as opposed to the minimum increase in heat flux, is difficult to estimate. However, a maximum temperature increase of 100 °C is suggested.

South America as a reference frame

To investigate this problem further we need a reference frame. The hotspot reference frame¹⁸ shows no relationship between possible hotspots or other hot areas and areas of uplift in southern Africa (Fig. 1b), presumably because hotspots do not cause plateau uplift. The hotspot reference frame is therefore rejected. Absolute reference frames are those in which there is no net rotation of the lithosphere¹⁹. Palaeomagnetic data suggest that for the past 50 Myr the equatorial components of any net finite rotation of the lithosphere relative to the mean global palaeomagnetic pole are perhaps as small as 2° (refs 20, 21). Because of the longitude indeterminacy, palaeomagnetic data cannot be used to find the polar component of any such rotation.

Nevertheless, I take here as reference frame that continent which has moved least relative to the global palaeomagnetic pole in the past 100 Myr, realizing that a finite rotation of arbitrary magnitude about the pole can be superimposed at any time on such a continent without being palaeomagnetically detectable. The continent that has moved least relative to the global palaeomagnetic pole is South America (Table 1)²². That

Table 3 Angular differences between instantaneous 'absolute' rotation poles and finite rotation poles for 55-Myr reconstruction

Fixed continent	Continent moved	Differences (deg) between finite rotation pole and instantaneous 'absolute' rotation pole			
			Model ¹⁹		
		B4	C4	D1	
Antarctica	Africa	37.8	32.7	51.9	
Eurasia		47.6	47.3	41.2	
North America		36.6	41.9	18.6	
South America		15.5	20.3	4.3	
Antarctica	Australia	22.8	24.9	24.0	
Eurasia		19.6	21.8	20.9	
North America		6.5	11.1	4.3	
South America		7.5	4.1	11.3	

is, its average distance from the pole and the average distance moved during successive intervals are smaller than that for any other major continent.

Independent support for taking South America as a reasonable approximation to an absolute reference frame is provided by absolute motion studies¹⁹ (Table 2). Except for the Caribbean plate, in five out of eight models¹⁹ examined, the American plate (combining North and South America into one plate) has the smallest r.m.s. absolute velocity¹⁹. South America alone would have the smallest r.m.s. absolute velocity of all the larger plates because it generally lies close to the American rotation pole (Table 2). Inspection of the velocity field for model A3 of Solomon et al.'s¹⁹ also shows the very small linear velocities of South America.

South America was adopted as a reference frame because it approximates to the present absolute reference frame, and the rotation poles support this choice. Lithospheric thickening and subduction slab pull seem to account for most of the driving forces on a plate²³. In the case of the Australian and African plates the gross relative geometry of ridges and subduction zones has not changed much in the past 55 Myr. Before then, Australia was part of the Antarctic plate and a very different plate geometry existed^{21,24}. Therefore, we might expect that, to a first approximation, the instantaneous rotation poles in the absolute reference frame are not far from the finite poles for the period 0–55 Myr. We know the finite poles relative to the continental plates²². The plate that moves least relative to the absolute reference frame is likely to be that plate with respect to which the calculated finite rotation poles of Australia and Africa are closest to their present-day instantaneous-absolute rotation poles.

The three absolute motion models¹⁹ closest to Harper's successful driving force model^{24,25} were chosen (B4, C4 and D1)¹⁹ and the results compared (Table 3). (D1 gives too high a net rotation of the lithosphere, but is retained for interest.) Clearly, the assumed finite pole of Australia in the absolute reference frame is equally close to the actual finite rotation pole in a reference frame fixed to either of the Americas. But only South America gives an actual finite rotation pole which is close to the assumed finite absolute reference frame pole for Africa.

During the period 100-0 Myr, Africa has rotated anticlockwise relative to South America (Fig. 1c, d). In particular, those areas affected by plateau uplift now overlie or have passed across the former positions of the oceanic ridges that separated Madagascar and India from Africa in the same period. There is, in fact, a remarkable coincidence between the area of high Africa and the area swept out by these oceanic ridges in a reference frame fixed to South America (Fig. 1a, c, d). The high-low Africa boundary essentially coincides with the trace of the southeastern edge of Africa at 80 or 60 Myr on presentday Africa (Fig. 1c, d). The hypothesis is that a thermal pulse, originating in the underlying asthenosphere at ~70 Myr, caused uplift about 50-60 Myr later. Imposing the small rotation needed to put South America into the present absolute reference frame (Table 2), assuming it has been unaltered for 70 Myr, would not alter this visual correlation between uplifted areas and former ridge positions.

Late Cenozoic uplift and volcanism of south-east Australia

I test this hypothesis by examining the uplift history of a second otherwise stable continent that also overrode former oceanic ridge positions in a South America reference frame: south-east Australia. Relative to South America, south-east Australia moved onto the former ridge position in the Tasman Sea shortly after it had opened between 76 and 56 Myr (refs 26, 27). If the Australian lithosphere is similar to Africa, I expect strong uplift beginning ~16 Myr ago.

The south-east Australian highlands form a 300-km wide tract rising to over 1,000 m above sea level, whose western boundary is roughly parallel to the trace of the western edge

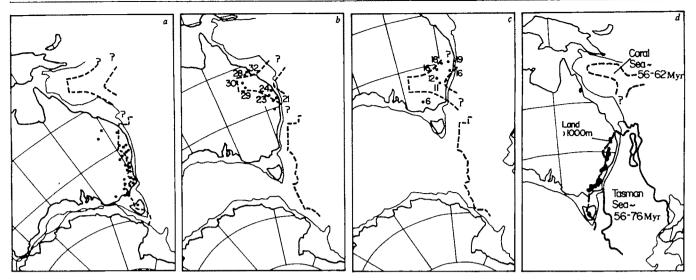


Fig. 2 a, Australia relative to South America at 55 Myr. The thick dashed lines show the approximate positions of the edges of Tasman Sea and the Coral Sea generated earlier relative to South America. •, Central volcanoes, ages in Myr (ref. 34), whose activity reached a peak in 55-34 Myr. Note the coincidence between these volcanoes and the position of the Tasman Sea ridge in a reference frame fixed to South America, with a time-lag of perhaps 20-30 Myr. b, As a but at 40 Myr. Note how the inferred position of the Coral Sea ocean floor at 40 Myr in a reference frame fixed to South America coincides with lava field provinces formed some 13 Myr later. In b and c, numbers are ages of alkaline central volcanoes in Myr (ref. 34). c, As a but at 20 Myr. Note how the inferred position of the Coral Sea ridge positions fixed to South America at 20 Myr coincides with the lava field provinces formed some 6 Myr later d, Present-day map showing Australia; the approximate age and distribution of ocean floor in the Coral Sea and Tasman Sea; the trace of the western edge of Tasman Sea from a and land over 1,000 m (black areas). Note the approximate coincidence of the highlands uplifted in Oligocene or later time, with the trace of the western edge of the Tasman Sea of early Tertiary age fixed to South America. a, b, c Are the same windows on Lambert equal-area maps in which South America is fixed; d is a window on a present-day Lambert equal-area map.

of the Tasman Sea at 76 Myr under Australia at 55 Myr, both in a South American fixed reference frame (Fig. 2d). Most studies conclude that at least in the south-east 80% or more of the uplift has taken place since the Oligocene (more recently than 22 Myr)²⁸⁻³¹, though some authors believe it to be much older³², or to have started earlier and to have been constant with time³³. Neighbouring areas have not been affected by so marked an uplift. Thus the area affected by uplift and the consensus of geomorphic interpretations fit well with the hypothesis.

Unlike high Africa, the south-east Australian highlands are closely linked to volcanism: a linear zone of basaltic lava fields is roughly coincident with the highland crest^{34,35} (Fig. 2a). Where data allow interpretation, a westward component is seen in the migration direction of this volcanism³⁴, which is expected if the heat sources are fixed in a South American reference frame.

At \sim 55 Myr, Australia started to move northwards relative to South America at \sim 53 mm yr⁻¹, eventually bringing the southeastern highlands region onto the former positions of the ridges in the Coral Sea, which was also relatively young³⁶ (Fig. 2b, c). A suite of alkalic central volcanoes formed, starting in the north at \sim 30 Myr, and migrating south to reach Victoria \sim 6 Myr ago, with an average migration rate of 66 ± 5 mm yr⁻¹ (ref. 34) (Fig. 2c). Like the basaltic lava fields, there is a similar time lag between the arrival of the affected parts of the highlands over the Coral Sea ridge positions and subsequent alkaline volcanism (Fig. 2b, c).

The similarity in timing between the lava field volcanism and the opening of the Tasman Sea has been noted previously^{34,35}, but no precise cause has been proposed. Likewise, the "remarkable migration of eruptive centres southwards (66 mm yr⁻¹) with time at a rate similar to the drift of Australia northward from Antarctica (56 mm yr⁻¹)" has been related to possible plumes and/or hotspots in the underlying asthenosphere^{34,35}. However, the northward migration of Australia relative to South America is at a similar rate, 53 mm yr⁻¹.

I propose that instead of asthenospheric plumes or hotspots causing magmatism, that the magma source originally lay within the Australian lithosphere. As a heat pulse moved upwards from the former positions of the Tasman and Coral Sea ridges,

the lithosphere thinned, eventually bringing the source region into the asthenosphere. As there seems to be a 20-Myr time lag between overriding of the continent and the onset of volcanism, I assume that the source originally lay well above the base of the lithosphere. Taking 65 Myr as the time constant for conductive heat transport for the whole continental lithosphere and a thickness of ~ 150 km, I estimate that a 20-Myr time lag probably implies a source depth of $\sim 70-110$ km below the surface.

The zone causing uplift some 60 Myr after Australia overrode the former positions of the western edge of the Tasman Sea must lie at much shallower levels, presumably in a zone capable of a phase change in the uppermost mantle. South-east Australian kimberlites, mostly of Jurassic age, include eclogites and other inclusions¹⁷. Refraction seismic lines show too that the present-day lithosphere in south-east Australia has a broad transition from crustal to mantle velocities, which could correspond to a zone of phase change³⁷.

Discussion

Provided that the former ridge positions also mark the locations of slightly hotter asthenosphere, the physical requirements of a phase change origin for African uplift lead to conditions that can be satisfied by the kinematics in a South American reference frame. I postulate that during the interval 80-40 Myr or so. what is now high Africa moved onto asthenosphere that had risen towards the surface as a direct result of Indian Ocean spreading. This asthenosphere was slightly hotter than the original asthenosphere under high Africa and created a heat pulse at the base of the African plate, which was too small to have a significant effect on the depth of the ocean floor, but was large enough to cause a phase change (or phase changes) in the uppermost 30 km or so of the continental mantle some 40-80 Myr later. Although three-dimensional mantle circulation models do not embody the complexities found in real-, cross-sections through such a circulation model in the present absolute reference frame at the present-day Carlsberg Ridge^{38,39} show the situation envisaged: deeper, and therefore hotter, asthenosphere rising up to replace preexisting asthenosphere under the edge of the African plate, which the plate will eventually cover. In the absolute reference frame the ridges must move relative to their immediately underlying asthenospheric sources 15

What is not clear is why the past positions of the ridges in the approximate absolute reference frame provided by South America seem to match the areas of uplift so well. One possibility is that because asthenospheric sources are probably vertically extensive^{38,39} and have horizontal velocities much less than the overlying plates^{38–40}, the asthenospheric zone extending from vertically above the deep source of a new ridge to the present position of the ridge may all have become hotter than it was before ridge creation. If so, this increased temperature could provide the heat needed for the phase change.

In detail, this model must be modified. For example, the uplift of southern Africa has been treated as a uniform vertical uplift simultaneously affecting all of high Africa, yet it is quite clear from Fig. 1c, d that we would expect a wave of uplift to have moved across high Africa starting in the east, and taking a geologically significant time to reach the west. This problem will not be discussed here in detail because part of its solution depends on considering still earlier phases of uplift probably resulting from the breakup of Gondwanaland. Nevertheless, it is worth noting that there is a distinct structural and topographic

Received 29 April 1981, accepted 27 January 1982

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asymmetry to southern Africa. The Cenozoic Kalahari basin lies in the western half of southern Africa, while the eastern half consists primarily of Precambrian rocks: during much of Cenozoic time, the eastern half of southern Africa has been structurally raised relative to the western half. In addition, the topography is markedly asymmetric: the highest parts of southern Africa are in Lesotho, very close to the eastern margin. Here, and nowhere else, the topography rises to well over 3 km. This structural and topographic asymmetry is what would be expected from a more detailed application of the model. The same mechanism is envisaged for the uplift of south-east Australia, where the expected topographic asymmetry is quite clear.

All these speculations can be tested by better data from the uplift of land surfaces, high-pressure petrology and threedimensional mantle flow models.

I thank P. England for providing a heat calculation programme and T. Holland for working out the likely values of the heats of reaction involved, also M. J. Bickle, E. Leitch, L. Nicolaysen, S. Richardson, F. J. Vine and N. H. Woodcock for comments and criticisms. Adele Prowse drew the figures. The production of the diagrams was greatly aided by the mapmaking system developed under a grant from the NERC GR3/2277. Cambridge Earth Sciences contribution no. 253.

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Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells

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DNA from T24, a cell line derived from a human bladder carcinoma, can induce the morphological transformation of NIH 3T3 cells. Using techniques of gene rescue to clone the gene responsible for this transformation, we have found that it is human in origin, <5 kilobase pairs in size and is homologous to a 1,100-base polyadenylated RNA species found in T24 and HeLa cells. Blot analysis indicates extensive restriction endonuclease polymorphism near this gene in human DNAs.

THE progression of a cell lineage from normalcy to malignancy may involve the mutation or activation of one or more genes. The genomes of retroviruses contain candidates for such 'oncogenes'. Certain retroviruses capable of inducing neoplasia in vivo and cell transformation in vitro contain transduced cellular genes which entirely encode the oncogenic proteins of these viruses^{1,2}. If these or other oncogenes are expressed in tumours of viral or nonviral origin, the introduction of these

genes into cultured cells might transform the recipients and render them tumorigenic. Indeed, DNA from some chemically transformed mouse cells can morphologically transform NIH 3T3 mouse fibroblasts following DNA-mediated gene transfer³. More recently, it has been reported that DNA from certain human tumour cell lines can also morphologically transform NIH 3T3 cells^{4,5}. We have detected transforming activity in DNA from 5 of 21 human tumour cell lines6; the resulting NIH 3T3 transformed cells are tumorigenic in nude mice⁴⁻⁶. Human DNA from one colon and two lung carcinoma cell lines appear to contain the same or highly related transforming elements, while DNA from a bladder carcinoma and from a neuroblastoma cell line each contain different transforming elements⁶. These transforming genes may have played a critical part in the origins of the tumours from which these cell lines derive. Here, we report the molecular cloning and preliminary characterization of the transforming gene from the human bladder carcinoma cell line, T24 (ref. 7).

Strategy of gene isolation

In principle, a gene can be isolated whenever its transfer into an appropriate host results in the stable acquisition of a selectable phenotype, using either 'search' or 'rescue' strategies. In the former, a complete genomic library is constructed in plasmids, phage or other cloning vehicles, and individual members of the library are screened by DNA-mediated gene transfer. This approach has been used successfully to isolate genes from lower organisms⁸. The rescue strategy entails cleaving DNA from cells containing the gene with a restriction endonuclease and then ligating this DNA to a defined 'marker' sequence. The ligated DNA is then used in gene transfer experiments, ultimately yielding a cell bearing the transferred gene linked to the marker sequence. The gene is isolated by one of several means on the basis of its association with the marker. The chicken thymidine kinase (tk) and hamster adenine phos-

Table 1 Transfection of NIH 3T3 cells with uncleaved and restriction endonuclease-cleaved DNAs from T24 and T24-a2 cell lines and recombinant bacteriophage and plasmid

а	Suppressor rescuant	Foci per 0.25 µg DNA
	λsup2.9	86
	λsup1.12	0
	λsup5.3	0
	λsup5.4	0
	λsup2.9	pTB-1
b	(Foci per 0.25 µg)	(Foci per 0.05 μg)
Undigested	86 (1.5×10 ⁻⁸)*	$372 (1 \times 10^{-7})^*$
BamHI	35	NT
BglII	71	NT
EcoRI	176	NT
HindIII	91	NT
XbaI	0	NT
	T24	T24-a2
	(Foci per 70 μg)	(Foci per 20 µg)
Undigested	$11(5\times10^{-7})^*$	$6(9 \times 10^{-7})^*$
BamHI	15	8
BglII	7	4
EcoRI	7	34
HindIII	13	10
XbaI	0	0

DNA (total concentration 30 μg ml⁻¹) was precipitated with calcium phosphate and 1 ml of suspension was applied to 10⁶ NIH 3T3 cells in a 10-cm culture dish, as previously described¹². Restriction endonuclease-digested T24 and T24-a2 DNAs were mixed in a 1:2 ratio with uncleaved NIH 3T3 DNA before precipitation; samples of phage and plasmid DNAs were added to 30 μg NIH 3T3 DNA before precipitation. Cellular DNAs were prepared by SDS-proteinase K lysis and phenol/chloroform extraction⁶. Phage DNAs were prepared from CsCl gradient-purified virions¹⁸ and plasmid DNA was purified from saturated culture of bacteria¹⁹. a Shows the NIH 3T3 focus-forming activity of DNAs from four independent suppressor-containing phages; b compares the focus-forming activity of λsup2.9 DNA and pBR322 subclone pTB-1 DNA, and compares the restriction endonuclease sensitivity profile of the activity in λsup2.9 DNA with the previously reported activities in T24 and T24-a2 cellular DNAs. NT, not tested.

* Value in parentheses is the calculated frequency of focus induction per transforming gene equivalent. In these calculations, we have taken the molecular weight of the human haploid genome to be 1.5×10^{12} .

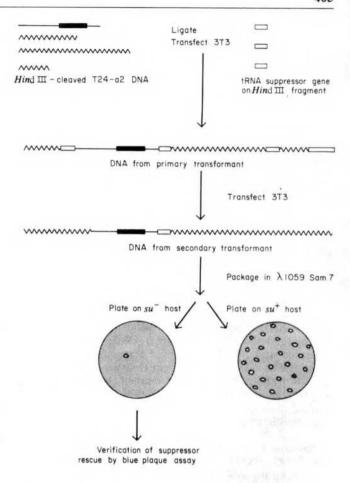


Fig. 1 Suppressor rescue of the T24 transforming activity. A recombinant DNA plasmid, pK-7, containing a supF tRNA amber suppressor gene was given by Dr U. RajBhandary. The suppressor gene was further subcloned into pBR322 plasmid (K.S. et al., in preparation). This subclone, pK-5, was cleaved with restriction endonuclease HindIII, and the 1.1-kbp suppressor DNA fragment was purified by gel electrophoresis and binding to glass powder²⁰ . T24-a2 is a transformed NIH 3T3 cell line derived from transfection of NIH 3T3 cells with T24 DNA. HindIII-cleaved T24-a2 DNA was ligated in fourfold mass excess to suppressor fragment DNA with T4 DNA ligase (New England Biolabs) at a total DNA concentration of 250 ug ml . Ligated DNA was mixed with a twofold mass excess of NIH 3T3 DNA and transfected to NIH 3T3 cells by methods described elsewhere 12. This ligated DNA induced foci at a threefold lower efficiency than native T24-a2 DNA in the absence of suppressor. Resulting transformed cells were cultured from individually picked foci. DNA from these cells should contain some supF sequences closely linked to the transforming element, but most supF genes, introduced by co-transfer, will not be closely linked. These DNAs were transfected again to NIH 3T3 cells, yielding secondary foci bearing only closely linked supF sequences. DNA from six secondary foci were partially digested with restriction endonuclease Sau3A, and 10-20-kbp fragments were purified by centrifuging 150 µg DNA through a 15-40% (w/v) sucrose gradient (in 100 mM NaCl, 10 mM Tris pH 7.4, 1 mM EDTA) at 23,000 r.p.m. in a Beckman SW27 rotor for 24 h at 20 °C. From the six individual size-selected DNA preparations, a total of 5.3 µg DNA fragments (250 µg ml⁻¹) were ligated to a twofold mass excess of gel-purified phage DNA arms generated by cleavage of λ1059Sam7 DNA with endonuclease BamHI. λ1059Sam7 was derived from in vivo recombination between \$1059 and \$Sam7 (K.S. et al., in preparation). Chimaeric phage DNA molecules were packaged into virions by a standard procedure¹⁵. A total of 8.4×10⁶ phage particles were generated by packaging, as measured by titration on E. coli BNN45 supEsupF cells. These phages were plated onto E. coli KS624 sup0(r-, m+) cells (K.S. et al., in preparation). Thirty-four individual plaques which arose were picked and the phages screened for the presence of amber suppressor by spotting on to lawns of $lacZ^{am}$ sup⁰ cells in the presence of lacZ inducer isopropyl thiogalactoside and indicator substrate, Xgal (5-bromo-4-chloro-3-indoyl-\$\beta-D-galactoside; ref. 21). Suppressor-bearing phage suppressed the $lacZ^{am}$ mutation, generating a blue plaque by β -galactosidase cleavage of Xgal. By this procedure, we isolated four suppressor-bearing phages. In the upper part of the figure, - denotes human DNA sequences; w. mouse DNA sequences; ■, T24 transforming gene; and □, tRNA supF gene.

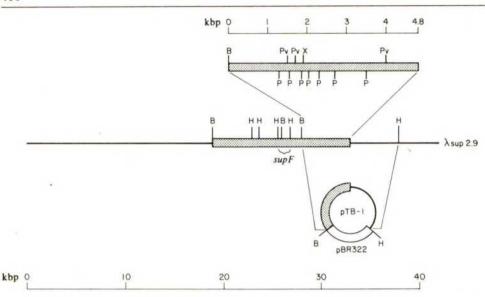


Fig. 2 Restriction endonuclease cleavage man of Asun2.9 and nTB-1. The figure shows the restriction map of DNA from the recombinant bacteriophage Asup2.9 which was active in transformation assays. Phage arms are denoted by a thin line, and the 14-kbp DNA insert by a shaded box. The suppressor DNA fragment' is marked supF. Below is shown the circular map of pTB-1, with the BamHI/HindIII fragment Asup2.9 cloned into pBR322, denoted by an open box. Above is shown a more detailed restricted map of the cloned T24 bladder carcinoma transforming sequences. The cluster of PstI sites were mapped by the method of Smith and Birnsteil²². There are no EcoRI, HindIII or BglII sites in this sequence. B, BamHI; H, HindIII; P, PstI; Pv, PvuII.

phoribosyl transferase genes were isolated using the Escherichia coli plasmid pBR322 as the marker sequence.

In this study, we have adopted a modified rescue strategy using an E. coli tRNA amber suppressor gene, supF, as marker. Details of this method will be published elsewhere (K.S. et al., in preparation) but the scheme is outlined in Fig. 1. From pK-7, a plasmid which contains supF, we constructed a new plasmid from which the supF gene could be recovered as a 1.1-kilobase pair (kbp) DNA fragment following cleavage with HindIII. This suppressor DNA fragment was ligated in equimolar ratio to HindIII-cleaved DNA from T24-a2, an NIH 3T3 transformant containing the T24 transforming gene. HindIII cleavage does not affect the transforming activity of either T24 or T24a2 DNA (see Table 1). We used T24-a2 DNA as our gene source because it has a higher specific activity of transformation than T24 DNA. Ligated DNA was mixed with a twofold mass excess of NIH 3T3 cellular DNA and added as a calcium phosphate precipitate to NIH 3T3 cells11,12. Morphologically transformed foci were picked 2 weeks later and grown into mass culture. Blot hybridization analysis indicated that cells from these foci contained multiple copies of the bacterial tRNA gene. Presumably, at least one of these copies directly flanked the transforming gene while the others did not. Therefore, DNA from these transformants was again used for gene transfer to generate new foci. DNA from a few of the resulting foci retained one or two copies of the tRNA gene, and these DNAs were used in subsequent experiments.

To clone the tRNA suppressor gene and its flanking sequences, we used a biological selection based on the ability of the cloned tRNA gene to suppress amber mutations when incorporated into a mutant of bacterophage A. We introduced by molecular recombination an amber mutation (Sam7)13 into the lysis gene of λ1059, a cloning vehicle which accepts 7-18-kbp DNA fragments having GATC3'OH cohesive ends14. The resulting phage, \$1059Sam7, required a supF host to complete a lytic cycle. DNA from transformed animal cells containing one to two copies of supF were partially digested with restriction endonuclease Sau3A and ligated to purified arms of λ1059Sam7 DNA generated by BamHI cleavage. The ligated DNAs were packaged into phage particles15 and plated onto sup⁰ and supF hosts. Phages which grew on sup⁰ hosts were further identified as supF-containing by their ability to convert a lacZ^{am} sup⁰ host to lacZ⁺. From such a screening process, we obtained four independent supF phage recombinants.

Cloned sequences transform NIH 3T3 cells

DNA from the four *supF* phages were mixed with NIH 3T3 carrier DNA and assayed for the ability to transform NIH 3T3 cells. As shown in Table 1, \(\lambda\)sup 2.9 DNA induced transformed

foci at an efficiency of ~ 300 foci per μg . The three other phage DNAs were inactive in transformation. As a first step in verifying whether the $\lambda \sup 2.9$ transforming activity is the same as that in T24 DNA, the activity of $\lambda \sup 2.9$ cleaved with restriction endonucleases was compared with similarly cleaved T24 DNA. Table 1 shows that the transforming activity of $\lambda \sup 2.9$ DNA is destroyed by cleavage with XbaI but not with BamHI, BgIII, EcoRI or HindIII. The same is true for T24 DNA.

To localize the transforming sequences of $\lambda \sup 2.9$, we subcloned restriction fragments of $\lambda \sup 2.9$ into pBR322. Cleavage

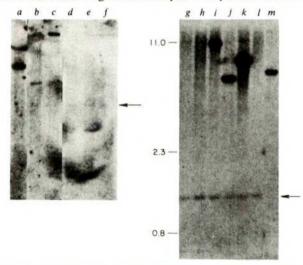


Fig. 3 Hybridization of pTB-1 DNA and one of its restriction endonuclease fragments to filter-blotted DNA from transformed NIH 3T3 cells. DNAs from NIH 3T3 cells, transformed NIH 3T3 and T24 cells were cleaved with either HindIII or BamHI restriction endonuclease. Digested DNAs (6 µg of each) were electrophoresed through 1.0% agarose gels and transferred to nitrocellulose filters as previously described 16. Blotted DNAs were hybridized with nick-translated 32P-DNA from either pTB-1 or the 800-bp PstI fragment of pTB-1. Hybridizations were performed at 74 °C for 16 h in 6× SSC, $1 \times$ Denhardt's solution and 20 µg ml⁻¹ denatured salmon sperm DNA²³. *Hin*dIII-cleaved cellular DNAs probed with pTB-1 ³²P-DNA (20 ng ml⁻¹) (a-f); BamHI-cleaved DNAs probed with the PstI fragment 32P-DNA (g-m). d, l, DNA from NIH 3T3. Secondary transformants of NIH 3T3 transformed with T24 DNA: T24-a1-3 (c, k); T24-a2-1 (a, j); T24-a5-4 (b, i). e, h, Secondary transformant of NIH 3T3 transformed with DNA from Calu-1, a human lung carcinoma cell line. f, g, Primary transformant of NIH 3T3 transformed with DNA from SK-N-SH, a human neuroblastoma cell line. m, T24. Hybridization of probe to HindIII-cleaved Asup2.9 DNA and PvuII- or PstI-cleaved pTB-1 DNA provided molecular weight standards as indicated (in kilobase pairs).

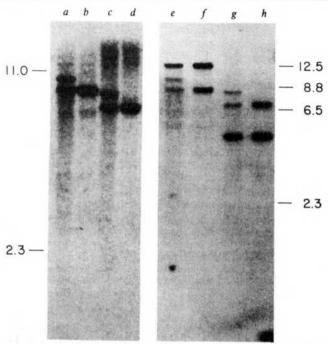


Fig. 4 Hybridization of pTB-1 DNA and its 800-bp PstI fragment to filter-blotted DNA from T24 cells and human placenta. Human placental DNA (given by J. Fiddes) and T24 DNA were cleaved with restriction endonucleases, electrophoresed through 1.0% agarose gels and transferred to nitrocellulose filters. Blotted DNAs were hybridized with 20 ng ml⁻¹ pTB-1 ³²P-DNA (e-h) or 2 ng ml⁻¹ gel-purified, ³²P-labelled 800-bp PstI pTB-1 DNA fragment (a-d). BgIII-cleaved placenta DNA (a, e); BgIII T24 (b, f); BamHI placenta (c, g); BamHI T24 (d, h). Hybridization of probes to XbaI- or HindIII-cleaved Asup2.9 DNA and PvuII-cleaved pTB-1 DNA provided molecular weight standards (in kilobase pairs).

of Asup2.9 DNA with BamH1 and HindIII generates seven restriction fragments, five of which contain heterogeneous ends (Fig. 2). These were then inserted between the BamHI and HindIII sites of pBR322 DNA. When individual plasmid subclones were tested for their ability to transform NIH 3T3 cells, only subclone pTB-1 had transforming activity, pTB-1 DNA contains a 9.8-kbp inserted sequence spanning the rightmost BamHI site in Asup2.9 to the HindIII site within the right arm of the phage DNA. As this region contains 5.0 kbp of λ DNA sequences, the transforming activity in pTB-1 is encoded in a transforming sequence no larger than 4.8 kbp. A more detailed restriction endonuclease map of this 4.8-kbp region is shown at the top of Fig. 2. The specific transforming activity of pTB-1 was higher than that of Asup2.9, but was consistently lower in a series of experiments than the specific activity calculated for the chromosomal gene in T24, assuming the gene is present in T24 as a single copy (for example, see Table 1). This agrees with our previous results for clones of the chicken tk gene which transfer at reduced efficiencies compared with the chromosomal gene9.

pTB-1 contains transforming sequences of T24 bladder carcinoma cells

Hybridization studies provide further evidence that we have cloned transforming sequences from the T24 bladder carcinoma cell line. DNAs from NIH 3T3 cells, NIH 3T3 cells transformed with T24 DNA and NIH 3T3 cells transformed with DNA from other human tumour cell lines were digested with *HindIII*, electrophoresed through agarose gels, and transferred to nitrocellulose filters by the procedure of Southern to nitrocellulose filters by the procedure of Southern transformants resulting from two cycles of transfection of tumour cell DNA through NIH 3T3 cells. The filters were hybridized with nick-translated 32P-labelled pTB-1 DNA. Figure 3 shows that pTB-1

sequences hybridize weakly to a specific HindIII fragment in DNA from NIH 3T3 cells and all its transformants (indicated by arrow at right of lane f). pTB-1 also hybridizes weakly to specific restriction fragments of normal rat cellular DNA (data not shown). In addition to these interspecies homologies, pTB-1 hybridizes strongly to a single HindIII fragment in each of three independent secondary transformants of T24 (Fig. 3, lanes a-c), while NIH 3T3 DNA shows no additional hybridization (lane d). We conclude that pTB-1 contains the transforming sequences present in T24 DNA. DNA from an NIH 3T3 secondary transformant derived from human lung carcinoma, Calu-1 DNA (Fig. 3, lane e) and DNA from an NIH 3T3 primary transformant derived from human neuroblastoma, SK-N-SH DNA (lane f), also lack additional sequences homologous to pTB-1, demonstrating that the transforming element in T24 differs from that in Calu-1 and SK-N-SH cells. This conclusion is consistent with our previous findings6.

Human placental DNA contains sequences homologous to the T24 transforming gene

If the transforming activity in T24 DNA arose by the acquisition of exogenous (for example, viral) genetic information, then

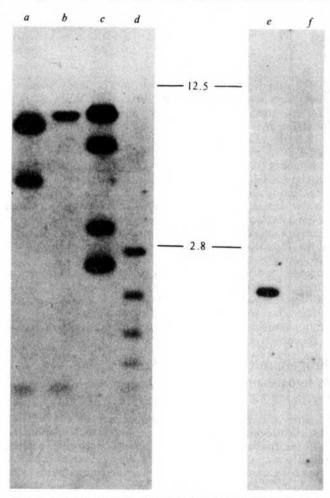


Fig. 5 Hybridization of pTB-1 DNA to filter-blotted T24 and pTB-1 DNAs cleaved with restriction endonucleases. T24 cellular DNA (6 μg) and pTB-1 (50 pg) were cleaved with restriction endonucleases, electrophoresed through a 1.0% agarose gel, then transferred to a nitrocellulose filter. pTB-1 DNA was cleaved with PstI (a) and PvuII (c); T24 DNA was cleaved with PstI (b) and PvuII (d). These blotted DNAs were hybridized to total pTB-1 ³²P-DNA (20 ng ml⁻¹). 20 pg of the 1.9-kb purified fragment of BamHI/XbaI-cleaved pTB-1 (e) and T24 DNA cleaved with BamHI/XbaI (f) were hybridized to the ³²P-labelled 1.9-kbp BamHI/XbaI pTB-1 fragment (5 ng ml⁻¹). Hybridization of probe to XbaI-cleaved Asup2.9 DNA provided molecular weight standards (in kilobase pairs).

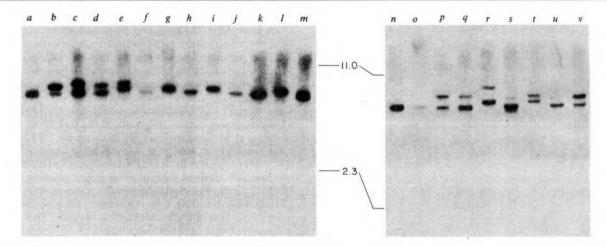


Fig. 6 Hybridization of the 800-bp PstI fragment of pTB-1 DNA to filter-blotted DNAs from human tumours and tumour cell lines. Human tumour cell lines were given by J. Fogh. Human tumours were obtained from the NCI Tumor Repository. DNAs from human tumours and tumour cell lines were cleaved with BamHI restriction endonuclease, electrophoresed through 1% agarose gels and then transferred to nitrocellulose filters. Blotted DNAs were hybridized to 2 ng ml⁻¹ gel-purified ³²P-labelled 800-bp PstI pTB-1 DNA fragment. DNAs from tumour cell lines: a, epidermis RPMI 2650; b, colon HT-29; c, cervix C4II; d, glial cell T98; e, osteosarcoma 8387; f, breast MCF-7; g, cervix HeLa; h, bladder T24; i, melanoma RPMI 4445; j, fibrosarcoma SW594; k, bladder 575A; l, bladder 496P; m, bladder TCCsup; u, pancreas Capan-1; and v, bladder VM-CUB-2. DNAs from human tumours: n, colon 031-10687; o, lung 031-9337; p, lung 031-11971; q, colon 76-640; r, lung 031-31317; s, colon 021-769; and t, lung 76-126. Hybridization of the probe to HindIII-cleaved λsup2.9 DNA and PvuII-cleaved pTB-1 DNA provided molecular weight standards (indicated in kilobase pairs).

pTB-1 would probably hybridize to a T24 DNA sequence absent from human placenta DNA. To examine this possibility, T24 and human placenta DNAs were cleaved with either BamHI or BglII restriction endonuclease, and digested DNAs were analysed by filter blot hybridization, using 32P-labelled pTB-1 DNA as probe. Figure 4 shows that pTB-1 hybridizes to 8- and 12-kbp BglII fragments (lane f) and to 5.0- and 6.8-kbp BamHI fragments (lane h) from T24 DNA. The probe hybridizes to BglII (lane e) and BamHI fragments (lane g) of identical size in human placental DNA. We also observed that T24 and human placental DNAs have similarly sized EcoRI or HindIII fragments homologous to pTB-1 (data not shown). As all the T24 DNA restriction fragments homologous to pTB-1 have similarly sized counterparts in placental DNA, we conclude that the T24 transforming gene originated from human genetic information without the gross rearrangements of 'normal' DNA sequences. The fact that additional 9.5-kbp BglII and 7.9-kbp BamHI fragments homologous to pTB-1 are present in placental DNA but absent from T24 DNA, we attribute to genetic polymorphism (see below).

Subgenomic fragments of pTB-1 suitable as hybridization probes for the T24 transforming gene

We wished to use pTB-1 as a hybridization probe to analyse the structure of the transforming gene in T24 and other human cell lines, and to monitor transcription of the gene in these cells. These applications require the human sequences in pTB-1 to be contiguous and not rearranged with respect to the chromosomal gene. Such rearrangements frequently occur after DNAmediated transfer into animal cells¹⁷. We therefore compared the restriction endonuclease map of the human sequences in pTB-1 with that of the homologous sequences in T24 DNA. Filter hybridization of T24 DNA cleaved with BamHI and XbaI to the 32P-labelled 1.9-kbp pTB-1 BamHI/XbaI fragment revealed a single homologous DNA fragment (Fig. 5f) comigrating with the 1.9-kbp pTB-1 fragment (lane e), proving that the region extending from the BamHI site to the XbaI site within the transforming gene is not rearranged in pTB-1. The 800-base pair (bp) PstI fragment of pTB-1 (Fig. 5a) mapping between 2.7 and 3.5 kbp from the BamHI site (see Fig. 2) can also be found in PstI-cleaved T24 DNA (Fig. 5b), and this PstI fragment hybridizes to DNA restriction fragments unique to NIH 3T3 secondary transformants of T24 (Fig. 3i–k). These results demonstrate that the 800-bp PstI fragment of pTB-1 is an unaltered component of the transforming gene or its flanking DNA sequences. However, the 2.3-kbp PvuII fragment of pTB-1 (Fig. 5c) spanning 1.7 to 4.0 kbp from the BamHI site is not present in PvuII-cleaved T24 DNA (Fig. 5d), indicating that human DNA sequences more than 3.5 kbp from the BamHI site in pTB-1 are rearranged. Thus, the 1.9-kbp BamHI/XbaI and 0.8-kbp PstI pTB-1 fragments were used as hybridization probes to study the structure and expression of the transforming gene.

Human DNAs are highly polymorphic near the T24 transforming gene

The restriction fragments of T24 and human placental DNAs homologous to the transforming gene were again compared, using the 800-bp PstI fragment of pTB-1 as hybridization probe. The PstI fragment hybridized only to the 8-kbp Bg/II fragment (Fig. 4b) and the 6.8-kbp BamHI fragment (land d) from T24 DNA. The probe detected identically sized Bg/II and BamHI fragments from human placenta DNA (Fig. 4a,c), although the cleaved placenta DNA additionally contains a larger homologous fragment in each case. The presence of these larger fragments indicates the existence of genetic polymorphism near the transforming gene.

To measure the extent of this polymorphism, DNAs from 40 human tumours and tumour cell lines were cleaved with restriction endonuclease BamHI and then analysed by gel electrophoresis and Southern blot hybridization, using the 800-bp PstI fragment of pTB-1 as probe. The data for 21 of these DNAs are shown in Fig. 6. Sequences homologous to the probe were found in DNAs from all human sources, but the number and sizes of the homologous restriction fragments differed among the DNA samples. BamHI-cleaved DNA from each human source contained one, two, or in one case, three discretely sized fragments ranging in molecular weight (MW) from 6 to 9 kbp. Among these DNAs, at least six different homologous BamHI fragments were evident. We have also detected the same extent of restriction endonuclease polymorphism among normal placenta DNAs from different individuals (data not shown).

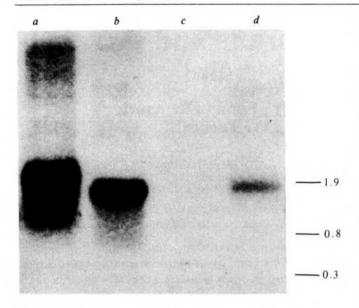


Fig. 7 Hybridization of restriction fragments of pTB-1 DNA to filter-blotted RNA. Total cellular RNAs were prepared by lysis of cells in 4 M guanidine thiocyanate²⁴ and removal of DNA and protein by extraction with hot phenol²⁵. Polyadenylated RNA was selected by oligo(dT)-cellulose chromatography; 5 µg of each poly(A)+ RNA were denatured in formaldehyde/formamide and electrophoresed through a 1.2% agarose gel containing 20 mM MOPS pH 7.0, 5 mM Na acetate, 1 mM EDTA, 2.2 M formaldehyde, and the RNA was transferred to a nitrocellulose filter, with 20× SSC as transfer buffer, as described by Thomas²⁶. The filter was hybridized at 42 °C in 5× SSC, 50% formamide, 10% dextran sulphate with 2 ng ml⁻¹ 800-bp *Pst*I pTB-1 ³²P-DNA fragment and 3 ng ml⁻¹ 1.9-kb *Bam*HI/*Xba*I pTB-1 ³²P-DNA fragment. Poly(A)⁺ RNA from 3T3 secondary transformant T24a2-1 (a), T24 (b), NIH 3T3 (c), HeLa (d). BamHI/XbaI-cleaved and PstI-cleaved pTB-1 DNA provide molecular weight standards (in kilobases).

The hybridization intensities of the homologous BamHI DNA fragments varied among the cleaved DNAs from different tumour sources. This variation is readily explained neither by inaccuracies of DNA concentration measurements nor by incomplete endonuclease digestions among the DNA samples analysed. We therefore suspect that the copy number of DNA sequences homologous to the T24 transforming gene varies about fivefold among the tumours and tumour cell lines.

Expression of pTB-1 sequences in transformed cells

To determine whether the transforming sequences are expressed in T24 cells and in NIH 3T3 cells transformed with T24 DNA, poly(A)⁺ total cellular RNA was prepared from T24 cells, NIH 3T3 cells, a T24 DNA-induced NIH 3T3 transformant and HeLa cells. The RNAs were electrophoresed in formaldehyde/agarose gels, transferred to nitrocellulose filters and hybridized with a 32P-labelled mixture of the 1.9-kbp Bam HI/XbaI fragment and the 0.8-kbp PstI fragment of pTB-1 DNA. The results are given in Fig. 7. NIH 3T3 cells transformed by T24 DNA contain heterogeneously sized RNA species (1,500-1,800 bases) homologous to the pTB-1 fragments (Fig. 7a), while untransformed NIH 3T3 cells show no homology (lane c). A homologous RNA of discrete size (1,100– 1,200 bases) is found in T24 cells (lane b).

The altered size of the transcripts found in NIH 3T3 transformants suggests that certain RNA processing signals in the transforming sequences were lost during gene transfer or are not recognized in the mouse cell host. A homologous RNA of the same size as that in T24 RNA is also found in the HeLa cells (Fig. 7d) but at reduced concentration. HeLa DNA does not transform NIH 3T3 in transfection assays. No homologous RNA species has been found in human placenta (data not shown). These results indicate that the transforming sequences are transcribed, that expression of these sequences is not restricted to T24 cells and that there is differential expression in cells from different sources. We estimate that transcripts of the T24 bladder carcinoma gene comprise 0.01% of poly(A)⁺ RNA from T24 cells.

Discussion

Previous studies using DNA-mediated gene transfer showed that DNA from T24 bladder carcinoma cells can efficiently induce foci of morphologically transformed cells in NIH 3T3 recipients⁶. In this study we have cloned from T24 DNA biologically active sequences which are responsible for transformation. Hybridization studies indicate that the transforming sequences contained in T24 are of human origin and closely homologous sequences are present in DNA from each of 40 different human sources that we have examined.

Of the 21 human DNAs screened, only 5 can efficiently transform NIH 3T3 cells⁶. T24 DNA transfers a different transforming element from the other four active donors; we conclude that the transforming sequences of T24 differ in some critical respect from the homologous sequences found in DNAs from other human sources. The transforming gene of T24 probably did not result from the gross rearrangement of normal sequences, as cleavage of T24 and human placental DNAs with BglII, BamHI, HindIII or EcoRI yields similarly sized fragments that are homologous to the transforming gene. However, the extent of restriction endonuclease polymorphism at this locus in human DNA limits the strength of this conclusion. Further studies should elucidate the origins of the T24 transforming gene and determine whether its biological properties derive from the expression of an altered protein product or from an altered pattern of gene expression.

We thank Dr J. D. Watson for support and encouragement; Drs U. RajBahndary, J. Karns, J. Kwoh and D. Zipser for useful suggestions and for supplying bacterial plasmids, phages and strains; and Dr J. Fogh for supplying human cell lines. M.G. is a Damon Runyon-Walter Winchell Fellow. This work was additionally supported by grants from the NIH and the Robertson Research Fund.

Received 31 December 1981; accepted 17 February 1982

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Structure of genes for membrane and secreted murine IgD heavy chains

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We have sequenced secreted and membrane terminal exons of the murine immunoglobulin δ chain. The putative transmembranal exon is strikingly similar to that of the μ chain and the internal cytoplasmic exon codes for exactly the same two amino acids, valine and lysine. A third potential exon was found between S and M exons that could code for yet another hydrophilic carboxyl terminus termed δX .

IMMUNOGLOBIN molecules exist in two forms: secreted (s) and membrane (m). Secreted molecules make up the neutralizing antibody population in the circulation and secretory fluids, whereas membrane immunoglobin molecules function as antigen specific receptors on lymphocytes^{1,2}. The majority of mature B lymphocytes strongly express both IgM and IgD on the cell surface with the same idiotype and ligand specificity, and hence, the same variable region³. When the B cell differentiates into a plasma cell upon receipt of an antigen dependent triggering, this same variable region is used for secreted antibody^{4,3}. Although the B cell population expresses surface IgM and IgD about equally⁶, the level of secretory IgD in plasma is about 1,000-fold lower than IgM^{7,8}. This may be due in part to the lower stability of IgD but it also must reflect the fact that cells committed to secretion of IgD must either be quite rare or must secrete δ in small amounts.

The simultaneous expression of two receptors on the B cell surface has led to several theories regarding their differential function. One proposal suggests that membrane IgD may deliver a triggering signal to the B-cell interior whereas membrane IgM provides a tolerizing signal. Another hypothesis holds that membrane IgM conveys a proliferative signal whereas membrane IgD provides a differentiative signal that allows the stimulated B cell to mature to an immunoglobulin secreting plasma cell. Yet another hypothesis holds that an IgD signal be required specifically when T cells are involved in B-cell triggering. Finally it has been proposed that the signal delivered by IgD may be the same as for IgM. in which case the existence of two receptors might serve to improve antigen binding, for example, to antigens with differing epitope density. It is clear that membrane IgM and IgD are not completely interchangeable, for the effect of suppression of IgM bearing cells is a profound immune deficiency whereas suppression for IgD has only minor effects.

Both secreted and membrane immunoglobulins have the same basic structure, but they differ in the amino acid sequence at the carboxyl terminus of the heavy chain. Studies by Rogers et al. 17 and Early et al. 18 on the structure of the messenger RNAs coding for secreted and membrane IgM show that the 3' ends of secreted and membrane mRNAs code for alternative carboxyl terminal amino acids and that these two types of mRNA could result from alternative processing of primary transcripts generated from a single C_H gene. The membrane form of the μ chain has a 41 amino acid hydrophobic carboxyl terminus encoded from two exons located some 1.8 kilobase pairs (kbp) 3' of the $C\mu$ structural gene 18. The secreted form of μ chain bears a shorter hydrophilic carboxyl terminus coded adjacently to the exon coding for the terminal domain, $C\mu 4$.

Both the size and sequence of the two μ mRNAs have been correlated with protein structure data for membrane μ^{19} and secreted μ^{20} .

In the case of IgD, there is also evidence for alternative carboxyl termini and multiple mRNAs. We initially characterized four $C\delta$ exons based on fine structure mapping and nucleotide sequencing of a cDNA clone derived from RNA of the IgD secreting plasmacytoma, TEPC 1017²¹. We also examined $C\delta$ clones of mouse genomic DNA. We found that a distally located exon (DC) encodes a hydrophilic segment which is transcribed as the 3' terminus of the major 1.75 kb δ mRNA of the tumour. Dildrop and Beyreuther²² have shown by amino acid sequence analysis of the mouse δ chain that the carboxyl terminal amino acid sequence of the IgD secreted by the hybridoma B1-8.81 corresponds to the DC encoded sequence (see below). Later we²³ and Moore et al.²⁴ noted the presence of an additional expressed region approximately 1 kbp 3' of the DC exon. We later showed²⁵ that this region hybridized to two larger (2.9 and 2.1 kb) δ chain mRNAs. These species were minor components of the plasmacytoma δ RNA but major components of spleen δ RNA. We proposed that this general region of the genome, termed VDC (or very distally coded), encodes the membrane carboxyl terminus. Maki et al.²⁶ obtained more extensive results and proposed a specific pattern of exons for the 3' end of δ membrane mRNA. Their study was based on the analysis of RNA from hamster-mouse and switch variant hybridomas by examining r-loops between RNA molecules and genomic δ DNA clones. In an IgD secreting hybridoma they found RNA similar in structure to the TEPC 1017 secretory form with the DC terminus. Additionally in both cell types they found membrane form RNA containing sequences from the VDC area with two types of 3' terminal exonic arrangements.

In this article we present a complete DNA sequence analysis of carboxy terminal δ exons correlated with a parallel RNA hybridization study of expression using normal spleen and plasmacytoma RNA as probes. On the basis of these results and those of Maki *et al.*²⁶ and the accompanying results of Fitzmaurice *et al.*²⁷ we present a specific proposal for the structure and RNA splicing patterns of δ mRNA.

Topology of the $\mu\delta$ area

Figure 1a, b presents a scale map of the entire $\mu\delta$ gene complex showing the four exons of the μ constant region, the two exons of the membrane terminus of μ , the three exons of the δ constant region, and the DC and VDC areas located downstream of $C\delta 3$.

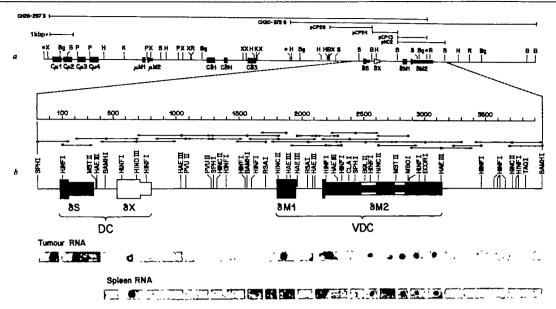


Fig. 1 Map of the $C\mu\delta$ gene complex. a, Restriction map of DNA coding for μ and δ genes is shown. The lines at the top indicate the genomic and pBR322 subclones used for sequence analysis. The exons are indicated by black boxes. Location of all $C\mu$ exons and the structural exons of $C\delta$ ($C\delta$ 1, $C\delta$ H, and $C\delta$ 3) in CH28-257.3 DNA were previously established stress represent end points of clones at Mbol sites. Other sites are abbreviated as follows: X, XbaI; Bg, Bg/II; B, BamHI; P, PsI; H, HindIII; K, KpnI; S, SphI; R, EcoRI. b, Restriction map of sequenced region spanning δ secreted (S) and membrane (M) exons. Boxes denote exons with the raised portion corresponding to sequences that are probably translated into amino acids. The lowered portions of boxes correspond to 3' untranslated regions. The strategy for sequencing by the chemical degradation method²⁹ is denoted by the dots and arrows: Fragments were cleaved and labelled at the dots either on their 5' or 3' ends, and the corresponding horizontal arrows give the direction and length of the sequence obtained; double-ended arrows represent sequences of strand-separated fragments. The scale above is given in base pairs. c, Localization of exons by dot-blot hybridization. Total RNA was isolated from TEPC 1017 or normal spleen, enriched for poly(A)⁺ mRNA by oligo (dT) cellulose chromatography and fractionated for size on sucrose gradients. These mRNA fractions were labelled with ³²P by polynucleotide kinase and hybridized to DC and VDC region DNA fragments immobilized on nitrocellulose according to Thomas³⁰. Autoradiographic results obtained from the critical experimental fragments are displayed beneath the corresponding map positions. The length of the fragments is indicated by vertical lines between the spots.

Two λ phage clones, Charon 28-257.3 and Charon 30-372.6 containing the δ region were isolated from shotgun collections of mouse liver DNA. Subsegments were cloned into plasmids for sequencing by the Maxam-Gilbert method²⁹. The endpoints of the various clones are indicated in Fig. 1a. An expanded map of the sequenced area is presented in Fig. 1b with the sequencing strategy delineated by arrows. The sequence covers approximately 4,100 base pairs (bp), most of which have been sequenced from both strands. Figure 2 shows the complete sequence including the translation into protein of regions that may be expressed, as well as other sites of interest.

Identification of δ exons

The pattern of expression of the sequenced area has been studied by three approaches.

- (1) Radioactive RNA from spleen and IgD secreting plasmacytomas was used as a probe for hybridization to DNA fragments immobilized on filters (Dot-blot method³⁰). These results are presented in Fig. 1c.
- (2) Radioactive fragments from genomic DNA clones were used as probes to detect mRNA species from spleen and tumours by the RNA blot hybridization method³¹. These results are presented by Fitzmaurice et al.²⁷.
- (3) r-loop data presented by Maki et al.²⁶ were scaled up by 10% and superimposed on the sequence with 3' termini aligned with poly(A) addition sites.

As shown in Fig. 1, the sequenced region displays two major zones of hybridization corresponding to the DC and VDC regions. There is no evidence for expression of mature δ mRNA within the approximately 1,000 bp separating these zones, nor 3' to the VDC region, nor 5' between the DC and the C δ 3 domain.

There are two potential exons in the DC area, which are termed δS and δX . The carboxyl terminus of δ secreted by tumours is coded by δS . The region denoted δX seems to be strongly expressed at the RNA level in dot-blot experiments

(Fig. 1c). In the VDC area we find two exons, δ M1 and δ M2, which encode the membrane carboxyl terminus of δ . An intervening sequence occurs in δ M2 in some RNA molecules (Fig. 1c and ref. 26). However, the position of the intervening sequence as measured from r-loops disagrees somewhat with our conclusions based on dot blots. We will discuss each of these exons in turn.

Carboxyl terminal exon of tumour-secreted δ

The carboxyl terminal 21 amino acids of the δ chain produced by myeloma and hybridoma tumours are encoded on the exon located 4.7 kbp downstream of the terminal structural domain, C δ 3. As detailed in the legend to Fig. 2, the genomic DNA sequence differs slightly from the TEPC 1017 cDNA sequence that we published previously²¹ due probably to a reverse transcription artefact. The actual B1-8. δ 1 protein sequence is identical to that which would be coded from the genomic sequence. It is not homologous to the secreted terminus of either μ^{20} or α^{32} .

Within 10-30 nucleotides 5' to the 3' terminal poly(A) tail of all eukaryotic mRNAs, the sequence AAUAAA³³ and, in rare cases AAUUAAA^{34,35}, occurs. An AATAAA poly(A) site which presumably signals the 3' end of δ s mRNA is located 163 bp downstream of the end of the protein. Assuming the actual end of the message is about 20 bp beyond the poly(A) addition site, that 200 A residues are added as a 3' tail and that a typical V region plus leader with 50 nucleotides of 5' untranslated region is present, the calculated length of the full δ s mRNA would be about 1,650 bases. This is in acceptable agreement with estimates of 1,700 to 1,800 bases by gel measurement^{23-27,36}. Likewise, the molecular weight for the secreted, nonglycosylated polypeptide chain calculated from the predicted amino acid sequence coded by the mRNA is 43,800. This is within 1% (ref. 36) and 7% (ref. 26) of reported

TCAGTAATTACAAGATACAAATGATTGATAACCAAGACAGAGACAGTACATGGGATTAGACCTGAGGTACGGGAAAGGTTGGGAAAGGATTCATAGTGTC
CCTTGCTACAAGAAGTCCCAGAGGTTTGCAGGGTGTGAGCAGATTCTAACCTCCCCATTGGCAAGTCTTCTCAAGGCTGCTTCTGCTACTAGAGAACCC

&S: \ C Y H L L P E S D G P S R R 300 400 500 600 700 800 1300 1400 1500 1600 1700 GARANCETACCTGGTCTAGCTGGTCTTTCTGGGTCTTTAAGTAGTAACCTAACTCTGCACCATCCGTGTGGCAGTAACATAGATCCACATCAGGTTTG GATTCAGATATTCCAATCCCATGCAGACCAATCTATGTGTGAGATTCCAGGATCCAGCAGGCTGGGGCATGGGTTGAGGGGTAGGATTCTTTGGGGCAAA GTGGTTAGTAGGCACTTGGGACTGAAAGTGTATAAGTATTAGCGGGAGGAGGAGGAGGATCAAAACATTGCTGCTGGATGTAAAGCTGTACCTTCAGGT 1900 2000 2100 2200 ITCCCTCAACATGAAGCCAACTAAGAAGATACCTTCTATGCAGAGAGAAATGCCAAGGCCCTCCTCTCAATACCTGCTCTCCACCTAGACT 2600 2700 2800 3 UT Possibility 2
TGCATACCTACTCAGATGCACATAAATTTACAGCAGAGCCTTAGGTGACAGGAATGGGGACTGTGGTCATTTGAATAAGAACCCCATAGGCTCATATAGT 3100 3200 3300 3400 3500 3600 3700 3800 GTGGAGAGCAGGAGGGAGGATACATTAGGAGATAACATCTTCCCTTCACCATTCTGGCTATTCCCTCACTATTCCCCTTGATGAACTTGGATCC

Fig. 2 DNA sequence of the $C\delta$ region spanning the secreted and membrane exons. Acceptor RNA splice site junctions are shown as /; donors as \. Positions of introns within the M2 3' untranslated region consistent with the dot hybridization (Fig. 1) (possibility 1) and electron micrographic data²⁶ (possibility 2) are indicated. Poly(A) recognition sequences shown in lower case. The δS sequence given differs from the previously published²⁰ tumour cDNA sequence at positions 296 (deletion), 301 (A), and 360 (T) (see text). Putative amino acids coded by expressed regions are displayed directly above the first base of the corresponding codon in single letter code: Phe, F; Leu, L; Ile, I; Met, M; Val, V; Ser, S; Pro, P; Thr, T; Ala, A; Tyr, Y; terminator, .; His, H; Gln, Q; Asn, N; Lys, K; Asp, D; Glu, E; Cys, C; Trp, W; Arg, R; Gly, G.

size measurements of cell-free translation products of δ chain mRNA.

Possible 'mystery' exon downstream of tumour-secreted terminus

Dot-blot hybridization experiments with both spleen and tumour RNA probes revealed a strongly hybridizing sequence about 300 bp 3' of δ S (Fig. 1). The BamH1-PvuII fragment spanning this region (Fig. 2 nucleotide position 436 to 1,158) also hybridized in RNA blot experiments²⁷ to a minor 2.65 kb RNA present in TEPC 1017. Inspection of the DNA sequence of this region shows that it has signals which might be expected for an expressed carboxyl terminal exon: a consensus acceptor RNA splice site, an open reading frame, a termination codon and a poly(A) addition site. We term this potential exon $C\delta X$. It would encode a 21 amino acid hydrophilic peptide that might serve as an alternative secreted form.

A dominant feature of the δX region is a tandem repeat $(GA)_{16}$ followed shortly by $(GT)_6(AT)_4$ located between the presumed translation terminator and the poly(A) addition site. This repetitive DNA segment in $C\delta X$ may be involved in establishment of secondary structure in δ mRNA. A complementary repeat occurs within the intron separating the $C\delta H$ and $C\delta 3$ domains³⁸ and again in the intervening sequence between the $C\mu M2$ exon and the $C\delta 1$ domain^{28,38} (Fig. 1). These three sequences could interact to form a complex overall folding of the mRNA. Part of this presumed secondary structure was seen previously when clone 257.3 was denatured and examined on electron microscope grids^{23,28}. Such structures may influence the splicing efficiency of δS mRNA in normal B cells.

Transmembranal segment of δ

Examination of the DNA sequence near the beginning of the strongly expressed portion of the VDC region reveals an area of strong homology with the μ gene exon, μ M1, which apparently codes for the transmembrane portion of the IgM

receptor molecule 17,18. Figure 3 shows an alignment of μ and δ gene sequences in that area. In the 120 bp region coding for the most hydrophobic segments of the μ protein, the μ and δ nucleic acid sequences are over 60% homologous. Allowing for a two amino acid gap, 50% of the amino acids match and similar amino acids are substituted at corresponding positions. We conclude that this segment, which we term $\delta M1$, serves to bind IgD to the membrane. The acceptor splice site beginning the δ M1 exon appears to be somewhat upstream of the corresponding site for μ M1, and the amino acid sequence for δ is thus longer by 14 amino acids than for μ . This segment is not particularly hydrophobic and would probably lie on the outside of the membrane. We term this sequence of the protein the 'spacer'. Interestingly, a cystine occurs in this portion of the IgD protein which might serve in cross-linking IgD half monomers (one H and one L chain) to one another on the membrane as was suggested by Eidels³⁹ and Mescher and Pollack⁴⁰.

Cytoplasmic segment of δ

The $\delta M1$ exon has a donor splice site at a position corresponding exactly to the end of the $\mu M1$ exon. Although this could be used to splice to any of a number of acceptor sites further downstream or not used at all, the next exon according to our hybridization results (Fig. 1c), is a region beginning 220 bp further downstream. This area is homologous to the μ M2 exon and splicing to this acceptor would lead to a carboxyl terminal peptide consisting of just two residues, valine and lysine. These are precisely the same amino acids that are found in the membrane form of μ . There is very little direct evidence concerning the amino acid structure of membrane IgD. The present DNA sequence predicts a molecular weight of 47,500 for the unglycosylated polypeptide assuming a typical V_H region and leader segment. This length is within 2% of the estimate of 48,500 for in vitro translation products of δ membrane mRNA²⁶. This agreement would appear to rule out a cytoplasmic component greater than about 30 amino acids. McCune et al. 41 have repor-

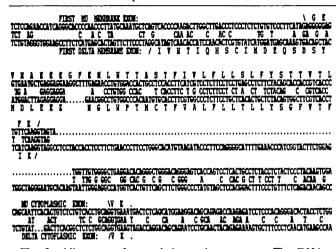


Fig. 3 Alignment of μ and δ membrane exons. The DNA sequence of the μ membrane coding region¹⁸ is shown aligned with the corresponding region of δ . Bases that match are repeated between the two sequences. Proteins coded from these DNA sequences are shown above and below using the single letter amino acid abbreviations. Regions that were gapped to bring the two

sequences into register are shown with dotted lines. RNA splice

sites are shown as slashes.

ted that for the human membrane δ chain no large cytoplasmic domain could be demonstrated by proteolytic digestion of *in vitro* products translated in a microsomal system. This is also consistent with the existence of only a small cytoplasmic protrusion

Beyond $\delta M1$ there are about 15 potential acceptor sites other than $\delta M2$, each of which, if used, would lead to a different carboxyl terminal peptide sequence. Moreover, a donor splice site occurs at the end of the $\delta M2$ exon coinciding with the protein terminator codon so that an array of additional peptides conceivably could be coded beyond the Val-Lys sequence. Since the number of possible mRNAs is quite large, a direct analysis of the membrane δ carboxyl termini at the protein level and analysis of many cloned mRNAs will be needed to establish whether any of the alternative patterns are used. This would be quite difficult if such an event occurs rarely or only during transient states of cellular differentiation.

3' End heterogeneity of δ membrane mRNAs

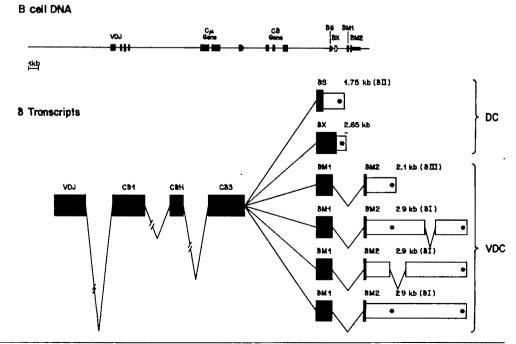
There are two positions at which poly(A) addition sequences occur which could signal the end of the mRNA for membrane

forms of IgD. These are an AATAAA located at nucleotide position 2,680 and an AATTAAA located at position 3,271 (indicated in lower case in Fig. 2). To ascertain whether these are the sites that are in fact used in vivo, two restriction fragments from the region were used as probes to examine mRNA by the RNA blot technique. The first fragment from the ClaI site at 2,568 to the MboI site at 3,035 covered the region between poly(A) addition sites. This DNA hybridized only to the 2.9 kb δ mRNA (ref. 27, Fig. 2). The BamHI (1,748) to ClaI (2,568) fragment derived from the region to the left of both poly(A) sites, hybridized to both the 2.1 kb and the 2.9 mRNA species (ref. 27, Fig. 2). All probes derived from DNA segments located downstream of the HaeIII site (3,303) were negative on both spleen and plasmacytoma RNA preparations. These results thus show that the 3' ends of the two major forms of membrane δ are correlated with the two poly(A) addition sites we have identified by sequence analysis. The simplest interpretation of these data is that the length difference between the two membrane δ mRNAs results primarily from the poly(A) termination site used, and that there is no difference in the protein coded by these two mRNAs. On this assumption the lengths of the two mRNAs can be calculated as 1,920 and 2,510 bases which is in reasonable agreement with the measured sizes of these RNAs. There is precedence for 3' heterogeneity generating multiple mRNAs which code for the same protein in the mouse dihydrofolate reductase gene⁴². The relative abundances of 2.9 and 2.1 kb forms of RNA²⁷ suggest a strong preference for the more 3' of the poly(A) sites. This may be due to the greater efficiency of AATTAAA as a poly(A) site. Secondary structure may also be responsible for this difference.

RNA splicing within the 3' untranslated region of δM

The r-loop data of Maki et al.²⁶ and the dot-blot data of Fig. 1 both indicate the presence of an intervening sequence near the end of the longer (2.9 kb) message. But the two methods suggest different positions for this intron. A model for the possible ways to express this transcription is presented in Fig. 4. On the basis of a computer search for donor and acceptor splice sites, either position for the intervening sequence could be accommodated. In the case of the r-loop data, a donor acceptor pair at 2,944 and 3,018 could be used (possibility 1 in Fig. 2), whereas a pair at 2,644 and 2,806 could be used to accommodate the dot-blot data (possibility 2 in Fig. 2). The latter possibility would be quite intriguing because it would lead to splicing out of the first poly(A) site from the mRNA

Fig. 4 Proposed RNA processing patterns that generate δs and δm RNAs. The upper portion shows a map of the $\mu\delta$ gene complex as arranged in B lymphocyte DNA. The lower portion shows six possible 8 mRNA transcripts with alternative carboxyl terminal exons. Regions of the exons (boxes) in black are translated and regions in white are untranslated. Thin lines denote RNA segments that are spliced out in the mature mRNA and black dots denote poly(A) recognition sites designations of Maki et al.26 for the RNAs are given in parentheses. Note that the same 8 amino acid sequence is obtained from all four δm forms, and that the three 2.9 kb forms differ only in the position (or lack of) the intron in their 3' untranslated region.



which could explain why the second site is used in that message. It is generally thought that polyadenylation precedes RNA splicing⁴³. However, this may not be obligatory in all cases. Thus in δ mRNA the rate of splicing could be rapid relative to that of polyadenylation. Another explanation consistent with our data is that a fraction of the 2.9 kb mRNA is cleaved to the 2.1 kb species and secondarily polyadenylated.

Regulation of δ expression

The μ - δ gene complex is a versatile genetic region which appears to be regulated by almost every known mechanism. Control of mRNA splicing and RNA chain termination are clearly crucial to this regulation, yet almost nothing is known of how this is controlled. Selection of splice sites is precise in the $\mu\delta$ transcript so that exons of μ never are reassorted with those of δ , yet the alternative carboxyl terminal structures of either chain can be simultaneously expressed or shifted in response to cellular signals. Clearly the point of mRNA termination is an important factor in determining where to splice since it can result in elimination of splice sites from the mRNA precursor molecule. We suggest that secondary structure of the RNA, caused by pairing of the repeated sequences we have observed, may also be an important factor in mRNA splicing since these sites could also be eliminated in response to changes in the poly(A) site selected. Thus a considerable degree of control, both quantitatively and qualitatively, could be exerted by alteration of the overall level of transcription termination enzymes in the B cell, ranging from low in the $\mu^+\delta^+$ B cell to high in the IgM secreting cell.

A more subtle regulatory effect is suggested by the phasing of RNA splicing in relation to translation. In both μ and δ the splice from the M1 to M2 exon occurs in exact register with the translation phase. This is in contrast to all other splicings known in immunoglobulin mRNAs where the splice occurs after the first base of the codon register. The use of a different phasing relationship for the cytoplasmic side of the membrane may create a barrier to reassortment of interior with exterior domains, since this splicing would create problems of translation reading frame.

Role of secreted forms of IgD

IgD is different from all other immunoglobins in that both secreted and membrane termini are encoded on separate exons downstream of the body of the C_H gene. Another major difference between IgD and all other Ig's is the lack of a switch-recombination type sequence between μ and δ^{44} that could efficiently create a secretory genomic arrangement by removing the $C\mu$ gene via the Honjo deletion mechanism⁴⁵. This correlates with the apparent lack of IgD plasma cells in young mice and their clonal appearance in old mice⁷. This suggested to us the possibility that IgD may be principally secreted from resting B cells. Considered in this light the IgD molecule may be more analogous to an antigen specific growth factor than to an ordinary antibody molecule. Clearly some efferent signal from the B cell is needed to account for the fact that in B cell-deficient (anti-µ suppressed) animals, antigenand idiotype-specific Lyt-1 bearing helper T cells fail to develop⁴⁶. In this role secretion of IgD could serve as a signal to the rest of the immune network, indicating the B cell population level that is expressing each V region and thus IgD may be central to maintenance of B cell homeostasis. The cessation of IgD production when the B cell differentiates either to an

Received 6 November 1981, accepted 8 February 1982

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IgM secreting plasma cell (increased RNA termination) or to an IgG or IgA secreting plasma cell (deleting δ) would both be entirely logical from a structional and functional point of

Do IgD and IgM deliver different signals?

It was to be expected that the general principle of membrane attachment that was demonstrated for μ would apply also to δ , but it is very interesting that the amino acids coded by δ M2 are identical to those of μ M2. This is the portion of the protein that would presumably extend into the cytoplasm and which could deliver a signal to the cell's interior. Our data predict that the signal delivered to the inside of the cell by $\delta M2$ coded IgD would be identical to that delivered by IgM-at least if the nature of the signal is determined by the terminal amino acid sequence exposed to the cytoplasm. However, it is possible that the entire immunoglobulin may be internalized as a result of antigenic stimulation, or other surface proteins (for example, Fc receptors or Ia molecules) may interact specifically with δ and not μ . In this case the two immunoglobins could deliver different signals despite the identical nature of the portions presented to the cellular interior.

Conclusions

With the completion of the DNA sequences of the terminal exons presented here, we have determined the complete structure of the murine $C\delta$ gene. The gene structure leads us to postulate the existence of three forms of the δ protein: δS , δM and δX . The existence of δX , however, remains provisional at present since it is based entirely on nucleic acid data. Our conclusions concerning δ transcription are summarized in Fig. 4. At least six mRNA species coding for δ have been discussed which differ only in their 3' ends. Four of them probably code for the same membrane protein (δM) although additional forms generated by splicing to alternative 3' coding segments cannot be ruled out in lieu of cDNA data.

The gene structure of δ is substantially different from all other immunoglobulins in that the $C\delta$ constant region has only two domains²¹ separated by a long, exposed hinge region. This may lead to the instability of IgD in serum, a feature that would be crucial if the secreted molecule (δS or δX) is to serve as an idiotype bearing chemical messenger as we suggested above.

In membrane δ , a 26 amino acid spacer region separates the last domain from the membrane anchor. This region is completely different for IgD, IgM and presumably for other classes. It thereby is a class-specific marker that can distinguish the immunoglobulin produced by its particular B cell from other irrelevant immunoglobulins that may be cytophilically adsorbed to the surface. In this role the spacer would be a good target for class-specific recognition by T cells or T-cell derived factors that promote maturation and differentiation of the immune response.

As for the antigen-specific receptor functions of IgD compared with those of IgM, the identity of the internal (cytoplasmic) amino acids suggests that the information pertinent to their differential roles does not reside on the inside of the B cell membrane.

This work was supported by NIH grants GM21812 (F.R.B.) and AI18016 (P.W.T.). We thank Joe Wells, Nancy Krowley, Al Zrolka, Chi-Ping Liu, Robin Robinson and Anita Gilliam for technical help, John Schroeder for computer programming and Daisi Marcoulides for help with the manuscript.

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IUE observations of variability and differences in the UV spectra of double quasar 0957 + 561 A, B

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Since the discovery of the double quasar Q0957+561 A, B by Walsh et al., their proposition that it represents a double image of a single object formed by an intervening galaxy acting as a gravitational lens has been amply confirmed. The intervening galaxy has been detected optically²⁻⁴ at a redshift of 0.36 (ref. 5). Further, a remarkable similarity in fine structure of the radio images has been demonstrated with VLBI observations⁶ and the required frequency invariance of the intensity ratio has been established from the UV through the optical and IR9 to the radio 10 regions of its spectrum. The first UV observations of the double quasar were made with IUE in December 1979, and have been supplemented by data obtained during September and December 1980. Here we present these new data together with an analysis based on the images being a gravitational lens effect.

The log of the new observations is given in Table 1. As previously, the nearby star HD237844 (B3, 9.4 mag) was observed as a comparison. This is necessary because there is some overlap of the two spectra of Q0957+561 A and B; these are separated by a deconvolution procedure which uses the profile of the instrumental point-spread function perpendicular to the direction of dispersion derived from the spectrum of the comparison star. The procedures for data extraction, deblending and correction for instrumental sensitivity are described elsewhere⁷

Because the object is faint (17th mag) for IUE, two separate observations have been made at each epoch and combined to improve the signal-to-noise ratio. For completeness, Fig. 1 covers the previous as well as the present data and shows the mean of two spectra, in 5 pixel bins (~11 Å), of component A (north) and component B (south) in each of the epochs 1979 December, 1980 September and 1980 December. Apart from the continuum, the only spectral features clearly present are the emission and absorption lines of Ly α . Their wavelengths are consistent with the respective redshifts $z_e = 1.41$ and $z_a =$ 1.39 given by Walsh et al.1

From the spectra in Fig. 1, the continuum flux in the range 2,500-2,800 Å and the emission flux in the Ly α (+N V) line were determined (see Table 2). It is evident that no changes have occurred in the spectrum of component B; the intensity of its continuum and $(Ly\alpha + N V)$ emission lines are the same, within the errors, at all three epochs. Component A, on the other hand, shows substantial changes. Its continuum level had fallen by a half by 1980 September and had almost recovered

to its 1979 December value in 1980 December. There is similar but less pronounced variation of the intensity of $(Ly\alpha + Nv)$.

Within the observational limits, the equivalent widths of the emission lines $(Ly\alpha + N V)$ in both components A and B were equal during December 1979 and December 1980 (see Table 2). During September 1980 the equivalent width of emission line in component B was similar (within observational limits) to equivalent widths in components A and B during December but the equivalent width of line in A had increased in the order of 50% compared with line in B, which was greater than the observational uncertainty. It is unlikely that the observed changes in the line and continuum intensity are due to instrumental effects because at the resolution of IUE, vignetting should affect both line and continuum intensities equally. The changes in the equivalent widths of emission lines also indicate that there is a phase lag between a change in the continuum level and the resultant change in the line intensity. Clearly the time grid is too coarse to measure this phase lag but an upper limit of 3 months can be established. This in turn gives an upper limit of 0.1 pc $(H_0 = 50 \text{ kms}^{-1} \text{ Mpc}^{-1})$ for the distance between the source of continuum radiation and the broad line emission region. This separation is an order of magnitude smaller than the separation assumed for modelling the broad line region¹¹.

The fact that the variation has occurred in A is important because any change in the source will be observed in this component first and should then recur in B after the appropriate time delay. To see what information may be available about this critically important parameter, our UV data have been combined with all other currently published intensity data; these are listed in Table 3 and plotted against time in Fig. 2. The range of frequency covered is very large, observations being made in the radio, IR, optical and UV. Where there is overlap there is reasonable agreement between the different observations. Some of the dispersion can be explained by the fact that the optical data include a contribution from the intervening galaxy which varies with wavelength, but the 1980 January observations⁵ at 4,000 Å seem more discrepant than expected from the errors. Over the months April-December 1979, the flux ratio shows no significant change and has the same value, within the errors, in all spectral regions from the radio to the UV. Beyond January 1980, the continuum flux ratio B/A at optical and UV frequencies starts to increase, reaching a maximum during August-September and almost recovers to its intrinsic value by December 1980. Unfortunately only UV observations reported here and available near maximum but the optical data taken immediately before¹² and after¹³ support the existence of the maximum. Table 2 shows clearly that the observed changes are confined to component A, both the continuum flux and $(Ly\alpha + N V)$ emission line flux of component B remaining constant within the error. This could be due to (1) a star in the halo of the lensing galaxy occulting only sight-line A14,15, (2) the lensed quasar being intrinsically variable, a change having propagated along sight-line A but not along B.

If a star in the halo of lensing galaxy occults a sight-line then radiation at all frequencies will be attenuated but line radiation

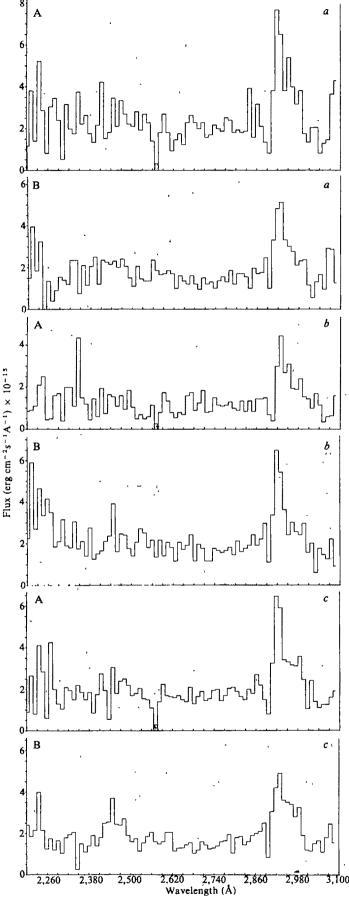


Fig. 1 UV spectra of double quasar 0957+561 A, B in 5-pixel bins (~11 Å). a, Mean of images LWR 6472/73 obtained on 24-25 December 1979. b, Mean of images LWR 8877/96 obtained on 23-26 September 1980. c, Mean of images LWR 9445/58 obtained on 6-8 December 1980.

Table 1 IUE observing log of 0957+561 A, B

Object	Image . LWR	Integration time (mm)	Date
0957+561 A, B	8877	350 .	23 September 1980
HD237844	8878	2	23 September 1980
0957+561 A, B	8896	305	26 September 1980
HD237844	8897	2	26 September 1980
HD237844	9444	2	6 December 1980
0957 + 561 A, B	9445	373	6 December 1980
HD237844	9457	2	8 December 1980
0957 + 561 A, B	9458	376	8 December 1980

will be attenuated less than continuum radiation as the source of line radiation is more extended than the source of continuum radiation14. At the resolution and signal-to-noise ratio of UV data it is not possible to estimate whether the line radiation has been attenuated more than the continuum radiation. On the other hand, if the lensed quasar is intrinsically variable then UV radiation is more likely to keep in step with visible radiation than with radio emission. The trend in the visible data is very similar to the trend in the UV data. Lacking the crucial radio data for September 1980, we conclude from the present observations that the lensed quasar is intrinsically variable but that during 1979 April-December the entire lens system was stable and no changes were propagating through this system and the observed mean ratio $B/A = 0.74 \pm 0.01$ represents the intrinsic ratio for the lensing system. Around August-September 1980 a change occurred in the lensed quasar and this change has been propagated along sight-line A but had not arrived along sight-line B by 1980 December and this allows us to place a lower limit on the delay time $\Delta T(B-A)$. At the 3σ level, changes have occurred in both the continuum and $(Ly\alpha + N V)$ emission fluxes of component A in 1980 early May and are still undetected in B in 1980 December. Hence,

 $\Delta T(B-A) > 0.6 \text{ yr}$

Current theoretical estimates of the time delay are very uncertain because it depends not only on the mass distribution in the lensing galaxy but also the mass distribution, both visible and hidden, throughout the cluster of which it is the brightest

Table 2 Continuum and emission fluxes and equivalent widths of emission and absorption

emission and absorption						
Image LWR	Continium	Huy (¥ 10 ⁻¹⁵ er	g cm ⁻² s ⁻¹ A ⁻¹)			
LWK .	A	B	B/A			
6472/73	2.0±01	1.5 ± 0.1	0.75 ± 0.06 .			
8877/96	1.0 ± 0.1	1.7 ± 0.1	1.7 ± 0.2			
9445/58	1.7 ± 0.1	1.5 ± 0.1	0.88 ± 0.08			
(Lya	+N v) emi	ssion flux (×10	$^{-13} \mathrm{erg} \mathrm{cm}^{-2} \mathrm{s}^{-1})$			
	Α	В	B/A			
6472/73	2.4 ± 0.1	1.8 ± 0.1	0.75 ± 0.05			
8877/96	1.5 ± 0.1	1.8 ± 0.1	1.2 ± 0.1			
9445/58	2.0 ± 0.1	1.9 ± 0.1	0.95 ± 0.07			
•	(Lyα	+N·∨) emission	ı line equivalent			
		vidth (Å) (obse				
		A	В			
6472/73	1	20±6	120 ± 10			
8877/96	1	50 ± 12	106 ± 8			
9445/58	1	18 ± 8	127 ± 9			
	Lyα	absorption equi	valent width (Å)			
		est-frame value				
		A	В .			
6472/73		2 (9.3)	8 4 (3.5)			
8877/96		7 (7.0)	9.5 (4.0)			
9445/58	,1	.8 (7.7)	10.3 (4.3)			
Mean EW	1	9 (8.0)	9.4 (3 9)			
Mean column density	1.2×	10 ²⁰ cm ⁻²	$2.8 \times 10^{19} \text{ cm}^{-2}$			

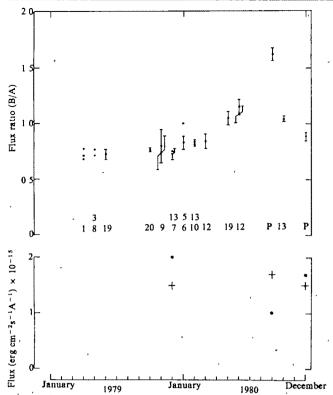


Fig. 2 The variation in the flux ratio QSO(B)/QSO(A) during 1979-80 (upper plot) and the measured UV continuum fluxes (2,500-2,800 Å) from QSO, A(●) and QSO, B(+). The numbers are references from which data were obtained; P, the present data.

member. Hence, from a grid of assumed positions for the cluster centre, Dyer and Roeder¹⁶ obtained time delays in the range 0.03-1.7 yr. With extensive new CCD observations of the surrounding cluster of galaxies and a more detailed modelling, Young et al.5 are able to fit the data with time delays of up to ~5 yr. Clearly, the observational limit given above restricts the range of possible models and continued monitoring to detect the same event in component B should give an actual measurement of the time delay. Because this scales inversely as the Hubble constant H_0 there is, in principle, the exciting possibility of measuring this out to z = 1.4, but uncertainties in the mass distribution of the lensing galaxy and surrounding cluster are too great to make an unambiguous determination of H_0 from a single time delay between two images. Young et al.5 point out that a good estimate of H_0 might be possible if two time delays, one between image A and B and a second between A. or B and as yet unobserved third image, were measured.

The equivalent widths of the Ly α absorption lines occurring at a redshift z=1.39, were also measured in the IUE spectra and are listed in Table 2 with the rest-frame values in brackets. As with the other measurements given in Table 2, these are made from the mean of two spectra taken of each component at three different epochs. These are reproduced in Fig. 3 but, unlike Fig. 1, they are unbinned and only cover the region of the Ly α line. An accurate estimate of the standard error is not possible in this case as a major uncertainty arises from drawing in a continuum in the neighbourhood of the emission component; we judge that the mean value for each component has an accuracy in the range 10-20%. The resulting column densities given in Table 2, derived on the basis of radiation damping, are therefore accurate to $\sim 20-50\%$.

Clearly the Ly α absorption in component A is greater by a factor of 2 than in B. The separation of the two light paths in the region of absorption is therefore a measure of the spatial structure in the absorption system. If we assume that the absorption system is at a cosmological distance then for $H_0 = 50 \,\mathrm{km \, s^{-1} \, Mpc^{-1}}$, a separation of $\sim 0.5 \,\mathrm{kpc}$ is obtained for the two absorption regions. The column density $\sim 10^{20} \,\mathrm{cm^{-2}}$ for H I

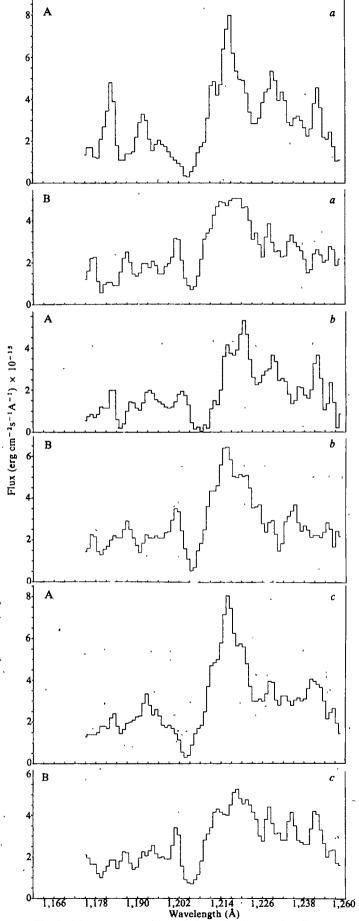


Fig. 3 Mean Ly α emission and absorption line profiles (in restframe) in a, December 1979; b, September 1980; c, December

Table 3	Observed	flux ratio	QSO(B)/QSO(SA)

	9 000 red rate (200 (B)) (200 (671)				
Date	λ	B/A	Ref.		
April 1979	3,500 Å	0.68	1		
April 1979	4,500 Å	0.70	1		
April 1979	5,500 Å	0.77	1		
May 1979	4,000 Å	0.76	3		
May 1979	4,000 Å	0.71	8		
June 1979	Radio	0.72 ± 0.04	19		
October 1979	Radio	0.76 ± 0.01	20		
November 1979	1.65 µm	0.80 ± 0.15	9		
November 1979	2.2 µm	0.74 ± 0.15	9		
December 1979	\mathbf{U}	0.75 ± 0.01	13		
December 1979	UV	0.72 ± 0.05	7		
January 1980	4,000 Å	1.00	5		
January 1980	Radio	0.83 ± 0.06	6		
February 1980	Radio	0.81 ± 0.01	10		
February 1980	U	0.83 ± 0.01	13		
March 1980	V	0.84 ± 0.07	12		
May 1980	В	1.05 ± 0.06	19		
June 1980	\mathbf{V}	1.15 ± 0.07	12		
June 1980	V	1.09 ± 0.07	12		
September 1980	UV	1.62 ± 0.06	Present data		
October 1980	U	1.04 ± 0.02	13		
December 1980	UV	0.88 ± 0.04	Present data		

in this region is higher than the values obtained for the halo of our Galaxy $(\sim 10^{19} \text{ cm}^{-2})^{17}$. If, however, the absorption system is intrinsic to the QSO and represents remnants of ejecta from the quasar then an upper limit to its distance from the QSO can be established by assuming that it has been moving at its relative velocity of $\sim 1,900 \text{ km s}^{-1}$ for $\sim 10^6 \text{ yr}$, the longest kind of lifetime expected for quasars¹⁸. This gives an upper limit on distance of ~2 kpc, which in turn gives an upper limit to the separations of the two absorption regions of $\sim 10^{17}$ cm.

We thank the staff of the IUE Observatory (VILSPA) in Spain for assistance and hospitality.

Following the submission of this paper, two reports^{21,22} of ground-based photographic photometry of the double quasar have appeared. Within the errors the intensity ratios B/A given in these papers agree with our results, that is they show a gradual increase up to June 1980 and a decrease from October 1980. (The maximum departure as revealed by IUE occurred during the intervening period when the double quasar was inaccessible to ground-based optical observations.) However, the two sets of optical data^{21,22} indicate that component A remained constant and that B varied, whereas our results indicate the exact opposite.

We believe that only further observations will resolve this contradiction. For any reasonable lens model for the double quasar³, events occurring in component A will be followed by similar events in B. Thus the optical observations indicate that variations in A must have occurred in the past, while our results indicate a forthcoming variation in B. The various lower limits on the delay time deduced from the respective data will obviously depend on which data are confirmed. We can predict that the UV and optical intensity of B will fade by a factor of ~2 over a period of months. This is best tested by continuing to monitor the object in the optical and UV; if positive, it will give an unambiguous measurement of the delay time.

Received 2 October 1981; accepted 19 February 1982.

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Asteroid rotation rates depend on diameter and type

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The rotational frequency of main-belt asteroids is shown here to depend on both asteroidal type and diameter. If asteroids of any one diameter are considered, then, on average, M asteroids rotate faster than S asteroids which in turn rotate faster than C asteroids. This shows that asteroids which have been classified by their surface properties alone have different bulk properties. For all three types, although the dispersions of the frequencies are large, we prove that the mean frequency increases linearly with the mean diameter. In both the C and S plots of mean rotational frequency against mean diameter there are discontinuities at diameters \sim 125 km and \sim 105 km, respectively, which may differentiate primordial asteroids from their collisional products.

Rotational frequencies of asteroids have been controversial ever since Alfvén¹ pointed out that all bodies except the Sun in the Solar System that have not been affected by tidal braking display an approximate isochronism of spin. Publication of opinions on the dependence of rotational frequency on asteroid diameter and type has since kept pace with the growing data.

McAdoo and Burns², for example, using a sample of 64 asteroids, claimed that smaller asteroids rotate more rapidly than larger ones. More recently, using a sample of 182 asteroids, Harris and Burns³ also concluded that there is a tendency for the smaller asteroids to rotate faster than the larger ones. However, they also concluded that there is little change in rotation rate with size when either C- or S-type asteroids alone were considered and that C-type asteroids appear to rotate ~80% less rapidly than the other asteroids. They consider that this, together with observational selection effects, could account for the apparent size dependence of the frequency distribution of the sample as a whole. (Various surface properties are used to separate asteroids into a small number of distinct groups. The chief difference between C and S asteroids is their albedos: C asteroids are much darker than S asteroids. For a complete discussion of the CSM asteroid classification scheme see ref. 9.)

Tedesco and Zappalà used data on 321 asteroids to define a set of 134 main-belt asteroids having reliable, photoelectrically determined rotational periods. Their criteria for inclusion in the set were designed to minimize observational selection effects and to isolate a set of asteroids with similar histories. With this set they were still unable to find any definite relationship between asteroid size and rotation rate but, in contrast to Harris and Burns, they recognized a group of large asteroids (diameter ≥175 km) that appear to rotate faster than the rest of the sample. They also found, like Harris and Burns, that S-type asteroids tend to rotate faster than C-type asteroids but concluded that the difference was statistically insignificant.

Burns and Tedesco⁵ concluded that there is no statistically significant size dependence for rotational frequency within either the S or C taxonomic type, but asteroids with diameters >175 km do rotate faster than the rest. They also consisder that a much larger data base is needed if any trends that do exist are to be discerned. We now show that this is not the

case. By analysing the same data set as that used by Tedesco and Zappalà⁴ we show that trends do exist in the data.

The data are shown in Fig. 1a: any trend is not apparent to the eye, but there is a significant linear correlation between rotational frequency and diameter. By ordering the chosen population in diameter and calculating running means, we can plot the mean rotational frequency against the mean diameter (Fig. 2a). In Fig. 2a the running box always contains 19 (= n) asteroids and was shifted through the chosen population one asteroid at a time.

The central limit theorem (see, for example, ref. 6) allows us to relate σ , the standard deviation of the distribution of the means of the samples, to σ_p , the standard deviation of the chosen population by

$$\sigma = \frac{\sigma_{\rm p}}{\sqrt{n}} f(N, n) \tag{1}$$

where n is the number of objects in each sample taken from a population of N objects, and

$$f(N, n) = \left(\frac{N - n}{N - 1}\right)^{1/2} \tag{2}$$

is a correction factor which allows for the fact that sampling

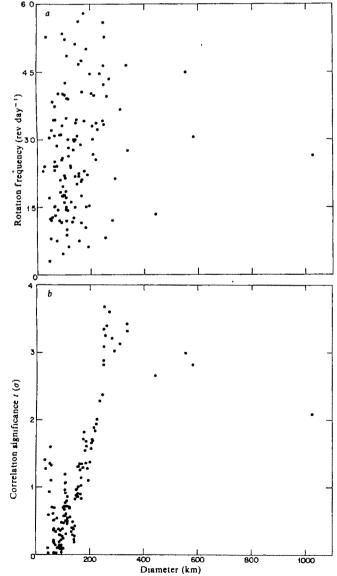


Fig. 1 a, Rotational frequency and diameters of 134 main-belt asteroids with reliable photoelectrically determined rotational periods. All data are from ref. 4. b, Variation of the statistical significance (σ) of the linear correlation coefficient with increasing diameter for the data shown in a. Note that the correlation is greatest for the 130 asteroids with diameters <400 km.

takes place without replacement: the first asteroid in the sample is chosen from N asteroids, whereas the nth is chosen from N-n+1.

Figure 2a shows clearly that rotational frequency increases with asteroid diameter. The mean rotational frequency increases linearly from a minimum of 2.1 rev day^{-1} (period = 11.1 h) for asteroids of diameter 60 km to a maximum of 3.7 rev day^{-1} (period = 6.5 h) for asteroids of diameter 230 km, that is, the mean frequency increases linearly from a point $2.0 \text{ } \sigma$ below the mean to one $3.5 \text{ } \sigma$ above.

The running box method gives a good description of the dependence of rotational frequency on diameter, but with this method it must be remembered that because each box contains 19 (=n) asteroids from a population of 134 (=N), then there are only $7 (\sim N/n)$ independent groups of data. Plots of the mean frequencies and the mean diameters of such independent groups for various values of n (19, 11 and 7) are shown in Fig. 2b, c, d. The uncertainties in the mean frequencies of these groups were estimated from the sample variances and in each case they compare well with the expected uncertainties estimated from the variance of the population as a whole.

We do not find any evidence of a discontinuity at diameter 175 km. Any trend that exists in the data seems to be present throughout the whole population. There is a suggestion, however, that mean rotational frequency does not increase indefinitely with mean diameter, but reaches a plateau of ~3.5 rev day⁻¹ (period ~7 h) at diameter ~230 km. However, because the population contains asteroids of different taxonomic types these values are not suitable for direct interpretation.

A useful measure of the significance of the correlation between rotational frequency and diameter is given by the linear correlation coefficient r. The statistical significance of r can be estimated from

$$t = r \left(\frac{N-2}{1-r^2}\right)^{1/2} \tag{3}$$

and if N is large ($\geqslant 100$) then the distribution of t can be assumed to be normal and t then represents the number of standard deviations (σ) from the mean. Figure 1b shows the correlation significance t as a function of increasing diameter D'. That is, the asteroids were ordered in diameter and t and t were calculated for those asteroids with diameter t of t with that t increases steadily with increasing t to some value t of the maximum is t of the maximum is t of the largest four asteroids in our chosen population causes t to decrease. In fact, if all asteroids including Ceres are included then t is reduced to t of the largest four causes t to decrease. In fact, if all asteroids including Ceres are included then t is reduced to t of the largest four causes t to decrease. In fact, if all asteroids including Ceres are included then t is reduced to t of the largest four causes t to decrease. In fact, if all asteroids including Ceres are included then t is reduced to t of the largest four asteroids including Ceres are included then t is reduced to t of the largest four asteroids including Ceres are included then t is reduced to t of the largest four asteroids including Ceres are included then t is reduced to t of t of the largest four asteroids including Ceres are included then t is reduced to t of
The file containing rotational periods in the Tucson Revised Index of Asteroid Data (TRIAD) is maintained by Tedesco⁸. Quality codes are used to indicate the reliability of the quoted data. Quality 1 indicates that the quoted period has been estimated from observations covering less than a complete rotation cycle. Quality ≥ 2 indicates secure results, that is, the rotational period is known to within a fraction of 1 h or better.

The 134 asteroids defined by Tedesco and Zappala are all of quality ≥ 1 , and include 48 S-, 45 C- and 9 M-type asteroids. Data describing the frequency (ω) and diameter (D) distributions, that is, the mean rotational frequency (ω) , the mean diameter (D) and the standard deviations σ_p of each subset of M-, S- and C-type asteroids are given in Table 1. Table 1 also contains data for those asteroids in the set of 134 with rotational periods of quality ≥ 2 . Running box plots of the data in Fig. 3a show that the tendency for mean rotational frequency to increase with diameter, which we have proved to exist in the data set as a whole, is not determined by the data in any one subset but is equally present in all three subsets of M-, S- and C-type asteroids: the tendency is a characteristic common to all major asteroidal types. Running box plots for the better quality data (quality ≥ 2) are shown in Fig. 3b.

Table 1 Asteroid data								
Subset	Туре	No.	$\langle \omega \rangle$ (rev day ⁻¹)	$\sigma_{ m p}$ (rev day $^{-1}$)	⟨ <i>D</i> ⟩ (km)	$\sigma_{ m p}$ (km)	m	ω_0 (rev day ⁻¹)
1	All	134	2.71	1.33	151.1	117.4		
··· i .	· C	45	2.47	1.31	193.6	148.7	0.013	0.24
1	S	48	2.57	1.08	117.5	55.8	0.014	0.73
· 1	M	, 9 -	3.28	1.75	113.6	62.4	0.023	0.63
2	· C	25	2.61	1.49	218.4	189.1	0.023	-1.65
2	S	· 32	2.80	-0.98	134.6	54.3	0.023	-0.17
2	M	6	3.85	1.78	128.2	72.6	0.024	1.08

Subsets: 1, photoelectrically observed, main-belt asteroids with rotational periods of quality ≥1 listed by Tedesco and Zappala⁴; 2, as 1 but only asteroids with rotational periods of quality ≥2. These subsets are further divided by taxonomic type.

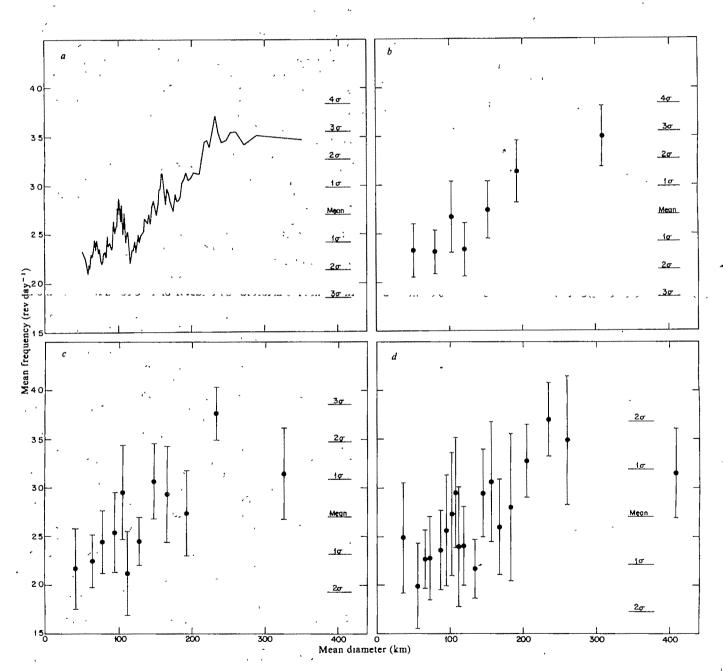


Fig. 2 a, A plot of the mean rotational frequency against the mean diameter for the 134 asteroids shown in Fig. 1 obtained by applying a running box method to the chosen population which were sorted in order of increasing diameter. The σ levels are derived by the application of the central limit theorem to the distribution of the mean of the samples of the population. The running box (and hence each sample) contained 19 asteroids and was stepped through the population one asteroid at a time. Mean rotational frequency clearly increases with mean diameter reaching a plateau ~3.5 rev day (period ~7 h) at mean diameter ~240 km. In b, c and d we show plots where the ordered data are shown in independent groups of b, 19; c, 11; d, 7 asteroids. The uncertainties in the mean frequencies were estimated from the sample variances and in each case they compare well with the expected uncertainties (shown at the sides) estimated from the variance of the population as a whole.

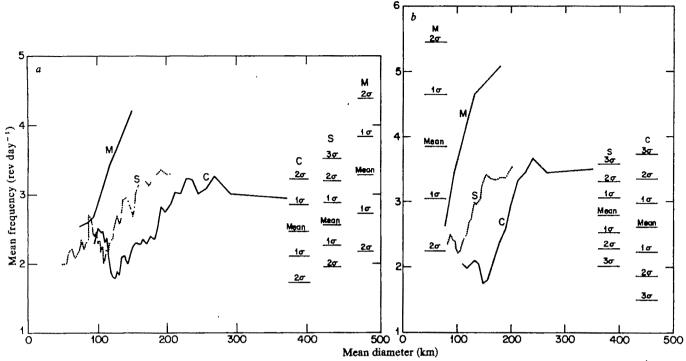


Fig. 3 A plot of the mean rotational frequency against the mean diameter for M, S and C asteroids listed by Tedesco and Zappalá and shown in Fig. 1, obtained by applying a running box method to the data which were sorted in order of increasing diameter. In a all data of quality ≥ 1 were included whereas in b only data of quality ≥ 2 were used. For the S and C plots the running box contained 10 asteroids. For the M asteroids the running box contained five asteroids in a and three asteroids in b. In all cases the box was stepped through the data one asteroid at a time.

The curves for each subset, but particularly those for S- and C-type asteroids, which are, of course, the best determined, are strikingly similar and contain several interesting features. Not all of these features are of strong statistical significance, but the fact that they are common to both the S and the C curves persuades us that they are probably real.

Each curve contains a near-linear portion for which we can write

$$\omega = mD + \omega_0 \tag{4}$$

Values of the slope m and of the intercept ω_0 for each nearlinear portion are given in Table 1. All the values of m, but particularly those derived from the better quality data, are very similar. In both the S and the C curves shown in Fig. 3b there is a suggestion of a plateau at mean frequency $\sim 3.\overline{4}$ rev day⁻¹ (period ~7 h). There are also significant discontinuities at small diameters. These are particularly evident in Fig. 3a which contains more data on asteroids with small diameters. The discontinuity on the C curve in Fig. 3a occurs at mean diameter of \sim 125 km at which point there is a 2σ increase in mean rotational frequency. A similar increase occurs on the S curve at mean diameter ~105 km. We consider that the observed increases in mean rotational frequency could be due to fragmentation of asteroids on collision and that the discontinuities may divide primordial asteroids from their collisional products.

The most important feature of Fig. 3, however, is that the curves for M-, S- and C-type asteroids are separate. If asteroids of any one diameter are considered, then, on average, M asteroids rotate faster than S asteroids which in turn rotate faster than C asteroids. As we have proved that rotational frequency does depend on diameter and because the M-, Sand C-type asteroids in our chosen population have different mean diameters, it follows that a simple comparison of the mean frequencies of the different subsets is of little value. We need a method which shows the dependence of rotational frequency on both diameter and type and the plots shown in Fig. 3 are ideal for this purpose.

We consider, but we cannot prove, that the discontinuities at small diameters and the plateaus shown in Fig. 3 are probably real. If this is the case, then the linear portions of the plots probably have the greatest significance. Using Fig. 3b and the data describing the linear portions of the plots given in Table 1, we can see that at any one mean diameter S asteroids rotate faster than C asteroids by a factor of 1.48 rev day⁻¹. For the S and C asteroids this represents a difference in mean frequency of ~2.6\sigma. Tedesco and Zappala5 found the same dependence on type for S and C asteroids but not the large statistical significance. However, they averaged their data over a very wide range of diameters and only compared the mean rotational frequencies of one group of asteroids from each subset. Our method allows us to examine the dependence of mean rotational frequency on mean diameter and on asteroid type in a single diagram and in such a systematic way that we can see that for all mean diameters S asteroids rotate faster than C asteroids. In fact, the separation of the curves is such that it makes better sense to state that if any one mean rotational frequency is considered, then, on average, C asteroids are significantly larger than S asteroids which in turn are significantly larger than M asteroids. (Note that the latter statement is not based on Figs 2 and 3, but on plots, not shown here, of mean rotational frequency against mean diameter derived from data ordered in rotational frequency rather than diameter.)

The running box method of averaging data has two advantages over a conventional histogram. (1) All points on a given curve are based on data for the same number of asteroids and therefore all points have equal weight. (2) The trend and structure in a given data set and some indication of their statistical significances can be displayed in a single diagram.

We thank Denise Gineris for help with the computations. This research was supported by NSF grant AST-802 4042.

Received 12 August 1981; accepted 15 January 1982.

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Resonant absorption by water polymers in the atmosphere

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There is much evidence both from atmospheric¹⁻⁹ and laboratory^{10,11} measurements that water vapour can show absorption of millimetre waves in addition to that predicted by models based on monomers¹² and dimers¹³. Constituents other than water can be eliminated as the cause of the extra absorption which is characterized by having a structured spectrum, increasing more rapidly with decreasing temperature than is expected from equilibrium phenomena and in the atmosphere showing a highly variable strength which is not simply correlated with total water content. The steep temperature dependence suggests invoking non-equilibrium water species to provide an explanation and this will account for variability as well as giving new prospects for understanding the spectral structure. A nonequilibrium model has, however, to be reconciled with what is known about supersaturation. It also opens the question of the nature of the energy source which is maintaining the steady state and allowing the extra absorption to be observed. I suggest here that water polymers containing about 50 molecules can, by collective acoustic resonances and postulated transition dipole moments, explain the observed component of absorption of millimetre waves by the atmosphere in some conditions. The polymers are metastable and are almost certainly the cause of supersaturation in cloud chambers but in these their lifetime is much greater than in the atmosphere.

Some examples of the structured spectra observed in high saturation and stable conditions given by natural fogs are shown in Fig. 1b, but the variability of the phenomenon causes most atmospheric observations to have poorer reproducibility than is shown by these. This has led some authors 14,15 to question the existence of anomalous absorption and to suggest that experimental artefacts could explain the effects. The data in Fig. 2 counter this by showing that measurements made by different experimental techniques used at different sites have an underlying common behaviour. They also show that this cannot be attributed to simple variations in the amount of water in the path. Low or zero values of anomalous absorption in some atmospheric conditions are to be expected and indeed were invoked to account for a few of the observations reported in ref. 5. The non-equilibrium nature of anomalous absorption also makes it difficult to interpret directly temperature dependence measurements specifically aimed at examining the role of equilibrium water dimers. There is, however, provocative evidence in finding high values of ~1 eV in the exponent of a Boltzmann factor describing results where the dimer binding energy would give ~0.2 eV. The high values are also supported by cloud chamber observations shown in Fig. 3 in which nonequilibrium conditions were deliberately created. The interpretation of these temperature exponent values will be discussed later.

To explain the spectral structure, the polymers are modelled as spherical liquid droplets which are assumed to show macroscopic surface tension behaviour. (Droplet is defined here as a liquid drop of such a size that an appreciable fraction of its molecules are on the surface. It may or may not contain a minority concentration of foreign atoms and may or may not be charged.) They will then have predictable collective acoustic modes with eigenfrequencies determined by droplet radius but if these are to be effective in electromagnetic absorption the major assumption must be made that the oscillations are accompanied by transition electric dipole moments. No values for

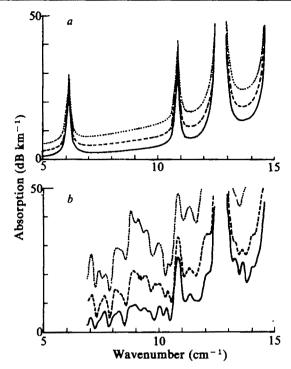


Fig. 1 a, Predicted spectra for fogs comprising molecular absorption and particle loss computed from standard models: corresponding to visibilities of 50 (···), 100 (---) and 150 (----) m. b, Spectra observed in the atmosphere corresponding to the models. The standard deviation in the measured spectra is 3 dB km⁻¹. In such fogs the liquid drops are all small compared with the wavelengths and show Rayleigh region behaviour in which attenuation varies monotonically. Neither this component nor equilibrium dimers can account for the observed additional structure between the monomer lines.

these are known but arguments for their existence can be given from considering the molecular structure of droplets of appropriate size. A molecular model for the polymers is also necessary to account for the complexity of the observed spectra. Classical macroscopic arguments applied to measurements of supersaturation are, however, sufficient to show that droplets in the required size range exist in cloud chambers and there seems to be no reason that droplets with the same size distribution should not be found in the atmosphere with a much reduced lifetime. This could be as small as 10^{-10} s and droplets could still be effective in millimetre wave absorption. Their existence in the atmosphere is supported by the measured temperature dependence of absorption giving high values of exponents.

dependence of absorption giving high values of exponents. The formulation of the theory uses results due to Kelvin-Gibbs (equation (1)), Frenkel¹⁶ (equation (2)) and Landau-Lifshitz¹⁷ (equation (3)) relating the quantities S = supersaturation ratio; $v_m = \text{molecular volume in liquid phase}$ (cm³); $\sigma_R = \text{radius corrected surface tension (erg cm²²)}$; R = droplet radius (cm); $R_c = \text{droplet radius at critical supersaturation (cm)}$; $G = \text{the droplet free energy at critical supersaturation radius } R_c$ (eV); $\rho = \text{liquid density (g cm³³)}$; $\omega = \text{acoustic capillary wave angular frequency in a sphere of radius } R$ (Hz); l takes integral values 1, 2... n with l = 2 the lowest value for the droplet to depart from sphericity; $\omega = 2\pi c\bar{\nu}$ where $\bar{\nu}$ is in cm¹ for electromagnetic waves as justified; c = speed of light (cm s³¹). The three relationships are plotted in Fig. 4.

$$\log_{e} S = 2\sigma_{\rm R} v_{\rm m} / R_{\rm c} kT \tag{1}$$

$$G = \sigma_{\rm R} R_{\rm o}^2 4\pi/3 \tag{2}$$

$$\omega = \sigma_{\rm R} l(l-1)(l+2)/\rho R^3 \tag{3}$$

The values of surface tension have been corrected for dependence on R by the expression proposed by Tolman¹⁸ and though controversial its inclusion does not affect the arguments in any

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critical way. The capillary waves are surface modes which for droplets will become the dominant acoustic eigenfrequencies. Further, as they describe motion of the liquid interface with free space and if accompanied by changes in dipole moment, the angular acoustic frequency can be related to electromagnetic oscillations using the free-space value for the speed of light.

If the curves of Fig. 4 are to be the basis of the kind of spectral structure shown in Fig. 1b, evidence must be given that some special significance can be attached to specific values of R. This comes in part from PVT observations¹⁹ in cloud chambers showing that if ions are not excluded a critical supersaturation ratio of about 4 cannot be exceeded but that at the critical value a long-lived state can be produced. Fig. 4a,b show that the corresponding specific droplet radius and free energy are $\sim 7 \times 10^{-8}$ cm and ~ 0.8 eV respectively.

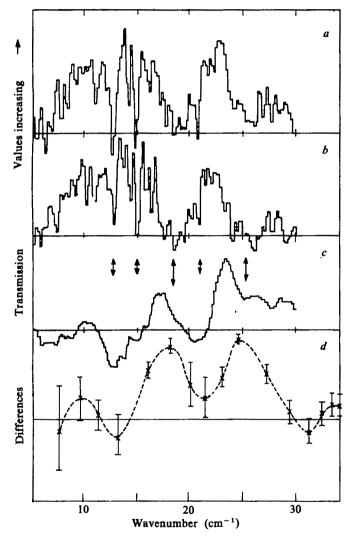


Fig. 2 a, b, Individual spectra of atmospheric emission measured successively within 30 min at Mount Evans, Colorado (see ref. 4). They represent relatively stable daylight conditions and show consistency in predicted water monomer and oxygen features but considerable variation at other wavenumbers. The difference between the two spectra shown by the smoothed curve of c has wavenumber maxima which do not coincide with the water lines indicated by the arrows and has both positive and negative values. It cannot therefore be attributed to varying amounts of water monomer. Curve d (derived from ref. 22) comes from a solar transmission spectrum observed at Gornergrat, Switzerland, with differences from prediction obtained by calibration with a black body. Again d has both signs and the resemblance to c which suggests that the cause of the differences is an atmospheric phenomenon common to the two sites and observable by different experimental methods.

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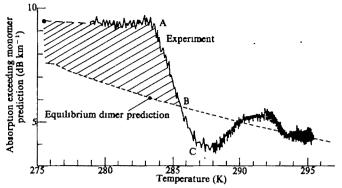


Fig. 3 Results on the temperature dependence of absorption of radiation in a band extending from 7 to 30 cm⁻¹ by a mixture of water vapour and argon. The gas had been expanded adiabatically to a supersaturation ratio of 2.5 in a cloud chamber and the solid line represents the observed changes in absorption on its relaxing to room temperature. This behaviour differs considerably from a model shown by the dotted line which is based on equilibrium dimers being the only addition to water monomers. The actual absorption is attributed to metastable droplets for which, using the assumptions in the text, a free energy of ~1.8 eV can be deduced by comparing the observed slope with the predicted slope at point B. This is based on dimer binding energy being 0.18 eV per molecule. The result is taken from unpublished work by W. B. Johnson et al. of Rutherford-Appleton Laboratory.

The latter represents an energy maximum which acts as a barrier to nucleation and maintains the unstable equilibrium. While there is a local minimum in droplet density at the critical radius there is also a local maximum in droplet lifetime which is an operationally important quantity in supersaturation though classical theory does not consider this. While it cannot be rigorously proven there is also reason to believe that the coincidence of long lifetime with the energy maximum is responsible for the measured temperature dependence of millimetre wave absorption giving characteristic energies of the order of 1 eV and thereby pointing to the existence of droplets in the atmosphere of about the same size. Although these energies came from equilibrium analyses it seems that the Arrhenius type plots^{11,20} used were indicative of reaction rates rather than energy levels but that these quantities are likely to be related.

While these arguments can provide a basis for a specific radius value and hence with Fig. 4c single peaks in the spectrum for each I value, the behaviour shown in Fig. 1b suggests that a more complex distribution of droplet free energy with size must be found. Classical macroscopic theory cannot provide this so results are given from a model which considers the effects of molecular behaviour within the droplets. The model is due to Plummer and Hale²¹ and their values of energy for droplets with discrete values of n between 40 and 57 are shown by crosses in Fig. 4b. These show large variations in droplet energy with radius or a kind of 'magic number' behaviour where Frenkel's relation is monotonic. Because droplet densities will vary exponentially with energy and it seems reasonable to assume that the variation in absorption cross-section with nwill be much slower, the Plummer and Hale values can be translated into changes in absorption with wavenumber which can be compared with observation. The calculated absorption change in going from n = 55 to n = 57 which corresponds to a spectral interval $\Delta \bar{\nu}$ of ~0.5 cm⁻¹ is ~60 dB km⁻¹. The maximum change of observed absorption in the same wavenumber interval in the region of 8 cm⁻¹ is ~15 dB km⁻¹. This shows that inclusion of molecular binding conditions can to a first order account for the observed spectral structure if the existence of transition dipole moments associated with the capillary wave oscillations can be justified. Also, from considering the molecular structure of droplets with $n \sim 50$ these moments are seen to be plausible. Macroscopic arguments require that both the total energy of the droplet and its surface energy be minimized and any molecular model must respect these conditions.

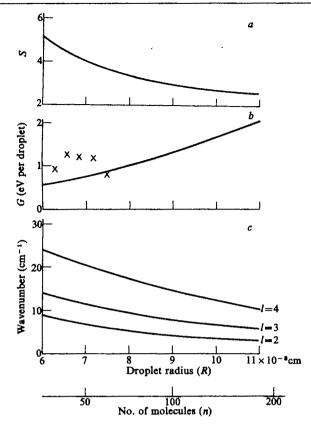


Fig. 4 Three quantities plotted against droplet radius R and the corresponding number of water molecules n. a, The supersaturation ratio S when the radius has the critical value R_{c} given by equation (1) (Kelvin-Gibbs). b, The droplet free energy, G for the critical radius, Re given by equation (2) (Frenkel) with the crosses representing droplet energy for specific n values of 40, 45, 50, 55 and 57 given by the model of Plummer and Hale²¹. c, With the assumptions of the text gives the eigenwavenumbers $\bar{\nu}$ for electromagnetic absorption by droplets of radius R according to equation (3) (Landau-Lifshitz).

It seems unlikely that an additional constraint can then be imposed on a given size of droplet containing only about 50 polar water molecules with marked steric properties that it should show no change in dipole moment when constrained to make the changes in shape with l value required by equation (3). The condition for complete absence of structured absorption is even more stringent in that the transition dipole moments would have to be zero for all values of n in the range of interest for all capillary modes. These incidentally have a degeneracy value of (2l+1).

The existence of metastable species raises the question of what are the likely sources of energy which are maintaining steady-state conditions. While no definite answer can be given as to whether the energy source is acoustic or electromagnetic in origin note that many of the observations in which the most marked anomalous spectral structure was observed were made in bright sunshine and that refs 22 and 23 are of particular interest in this connection. On the other hand in the laboratory measurements reported in ref. 11 no high energy photons were involved but the untuned resonator contained a source of acoustic power. In a purely acoustic experiment²⁰ high values of temperature exponent also suggest droplet formation.

The implications of the presence in the atmosphere of metastable absorbers and the need for energy sources to excite them greatly complicates the prediction of millimetre wave attenuation values. This complexity will almost certainly extend to other spectral regions²⁴ and will need to be considered in explaining observations. However, resonant absorption measurements could become a valuable new probe for the study of supersaturation.

An important feature of the present proposal is the introduction of collective acoustic oscillations and results on rough metal surfaces in the 4-12 cm⁻¹ range from Raman scattering have been explained in the same way²⁵. A generalized treatment of the onset of collective modes has been given by Fröhlich²⁶ and this may contain the means of removing, at a later stage, the ambivalence of the present proposal which comes from the simultaneous use of classical and molecular model results. A great benefit of using the Landau-Lifshitz capillary wave formulation is that eigenfrequencies are given in terms of macroscopic parameters and thereby a useful starting point is obtained. I know of no other experimental results using the formulation but its use has been proposed to predict the behaviour of globular proteins²⁷ in scattering inelastic neutrons and the scattering of light by superfluid helium²⁸.

I am supported by the Science and Engineering Research Council. Much of the work reported here was done at the Appleton Laboratory and I thank colleagues there for the use of results before publication.

Received 9 September 1981; accepted 3 February 1982

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Multi-armed vortices in an active chemical medium

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An excellent example of self-organization in nonequilibrium systems^{1,2} is the origination of rotating spiral vortices. These vortices have been observed in a wide range of active media-in the morphogenesis processes of social amoeba Dictyostelium discoideum^{3,4}, in cardiac muscle during some arrhythmias⁵ and in the Belousov-Zhabotinsky reaction⁸⁻⁹. All these vortices are simple spirals. The rotating structures of a higher order of symmetry such as multiarmed vortices have not previously been observed experimentally. We have obtained two-, three- and four-armed rotating spiral vortices in an active chemical medium. These structures were appreciably stable and we observed their rotation for more than half an hour, which was in striking contrast to unstable multiple vortices in many other physical systems (such as superfluid ⁴He or superconductors^{10,11}).

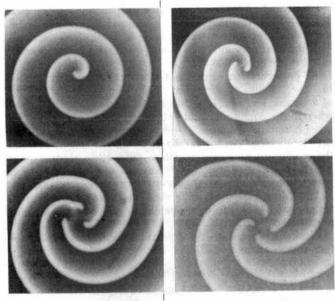


Fig. 1 One-, two-, three- and four-armed vortices in an active excitable chemical medium. A Petri dish 90 mm in diameter was filled with 4.5 ml of reagent similar to that proposed by Winfree: 0.3 M NaBrO₃; 0.4 1 M H₂SO₄; 0.1 M CH₂ (COOH)₂ 0.85 M CHBr(COOH)₂; 0.045 M ferroin.

The multiarmed vortices were obtained as follows. Two, three or four waves were initiated close to each other by touching the solution with a silver pin. Wave-breaks were then induced by stirring a little of the reagent with the pin. One drop of 1 M KCl was placed close to the wave-breaks, and the waves began to circulate around it as though it were an obstacle (Cl⁻ interferes with the oxidation-reduction chemistry¹²). After one or two rotations the drop was removed by a strip of filter paper, whereupon a multiarmed vortex arose. The number of its arms equalled the number of waves preceding its appearance. The

idea of this procedure was to begin with a regime which would be *a priori* stable, and then change to the regime we were studying.

To increase the accuracy of measurements and to avoid the influence of the small amount of Cl⁻ that remained in the reaction mixture, the vortex was displaced outside the domain occupied previously by the drop of KCl solution. This was achieved by passing through the solution a train of concentrational waves with a frequency exceeding that of the vortex. Thus, the vortex could be shifted without displacement of the liquid 13.

The vortices obtained are shown in Fig. 1. Far away from the core, the multiarmed vortices form equidistant rigidly rotating spirals in accordance with the well known solution (2) of the reaction—diffusion equation. But in the core region the wave behaviour has proved to be unexpectedly complex: there was no simple rotation; instead, waves were intermittently connected as shown in Fig. 2. The geometry and the kinematics of the waves in the vortex core would not fit the modern theoretical concept of vortex rotation (see equation (2)).

The periods of waves (T) sent into the medium by multiarmed vortices were found to increase with the number of arms: $T_1 = 28 \pm 1$ s, $T_2 = 29 \pm 1$ s, $T_3 = 31 \pm 1$ s, $T_4 = 34 \pm 2$ s (the mean from 7-15 experiments \pm s.d.).

All multiarmed rotating structures exist as long as the chemical reaction runs (>30 min). However, we also observed the decay of vortices. Figure 3 shows the disintegration of a two-armed vortex into two one-armed vortices. The disintegration was usually observed 50-60 min after mixing the reagents. Apparently, the disintegration was due to the change in active medium parameters with time. The two-armed vortices almost always decayed after 60 min of reaction life.

The decay of a three-armed vortex was observed in only one of seven experiments. However, in none of the experiments was the decay of a four-armed vortex evident. At the same time, all types of vortices were perfectly stable to external perturbations. The increase in temperature by 15 °C gave rise to a 2-2.5-fold decrease in the wavelength and the period, but

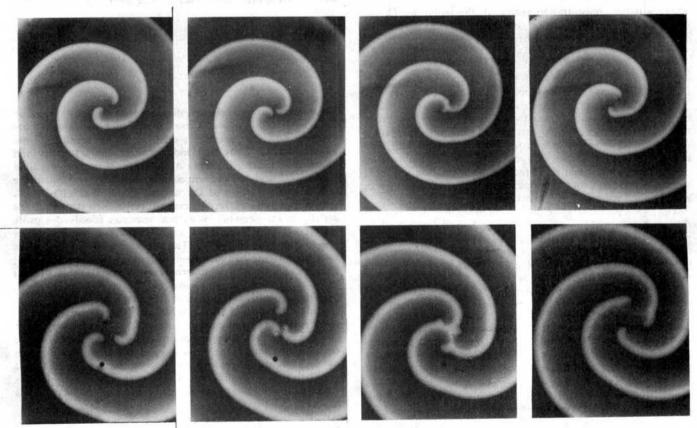


Fig. 2 Wave movement in the core of two- and three-armed vortices. Time interval is 15 s. Other conditions as in Fig. 1.

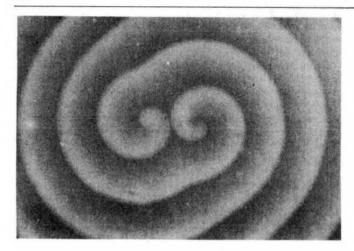


Fig. 3 Two closely spaced one-armed vortices formed from a two-armed vortex. The photograph was taken 58 min after mixing the reagents.

no signs of disintegration of multiarmed vortices were seen. In experiments where a multiarmed vortex was shifted by highfrequency concentrational waves, the structure of both the core and the outer region of the vortex was drastically changed, but in each experiment the vortex regenerated on switching off the external source of high-frequency waves.

Although similar structures to the multiarmed rotating vortices can be seen on some photographs of the aggregation of D. discoideum, these are not rotating waves. They are motionless and represent the path along which the cells are moving, whereas the form of the rotating concentration wave of cyclic AMP is a single one-armed spiral.

Active media are governed by the parabolic partial differential equation (the so-called reaction-diffusion equation):

$$\frac{\partial \mathbf{u}}{\partial t} = D\Delta \mathbf{u} + f(\mathbf{u}) \tag{1}$$

where \mathbf{u} is the concentration vector; Δ , the laplacian; D, the diagonal matrix of diffusion coefficients. The vortex-like solutions of this equation of the form

$$\mathbf{u} = F(N\theta - \omega t, r) \tag{2}$$

are known¹⁰ where r and θ are the polar coordinates, and N is the integer number or the topological charge of a vortex 10,11.

This solution describes the rigid rotation of a vortex with a constant angular velocity ω in a two-dimensional medium. Note that in a three-dimensional medium the centres of rotation form a thread—the axis of a vortex—which may be straight or curved 9,10 . The topological charge N characterizes the number of arms in a vortex and the direction of its rotation $(N = \pm 1,$ a one-armed vortex rotating clockwise or anti-clockwise; N = 2, a two-armed vortex; N = 0, concentric waves).

The topological charge of the system of vortices is conserved during their formation, disintegretion and annihilation both in physical systems and in active media-vortices emerge only in pairs with topological charges of opposite sign-a vortex with topological charge 2 may disintegrate into two simple vortices with topological charges 1, and so on.

Vortices with a topological charge N > 1 have been analysed with regard to the theory of superconductors, superfluid ⁴He and modern field theories ^{11,14}. As the vortex energy is directly proportional to N^2 , vortices with a topological charge N > 1are unstable in the physical systems considered. Similar results have been obtained by the theory of active media consisting of the van der Pol oscillators. It has been shown that unidirectional vortices repulse each other, which results in instability of vortices with N > 1 (ref. 10). These findings were the basis of the widespread opinion that the multiarmed rotating vortices cannot exist in real active systems because they must decay into simple ones as fast as formed.

How could we observe longlasting vortices with a topological charge N > 1? Note that energy is not conserved in active media. the waves propagate at the expense of local energy sources. Therefore, energy analysis, though successful in many other cases, is not applicable here. Linear analysis of the stability to small perturbations carried out after our experiments shows that the stability of vortices strongly depends on the kinetics of an active medium. In particular, for two-component media wherein the equilibrium state of each element is unstable, the quasi-harmonic solutions corresponding to vortices with a sufficiently large topological charge N are also unstable. But if the equilibrium state becomes stable, the vortices become stable for all values of N (ref. 15). Note that we obtained vortices with topological charges 2, 3 and 4 in a strongly relaxational excitable (not oscillating) active medium. However, no theoretical approach seems to be available to analyse vortex stability in that case.

We thank Professor A. M. Zhabotinsky and Dr A. M. Pertsov for valuable advice.

Received 19 July 1981; accepted 19 January 1982.

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Surface microlayer phenolic enrichments indicate sea surface slicks

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The realization by early investigators that oil films spread on surfaces of bodies of water could mimic slick conditions 1-3 has evolved into the present model of slicks resulting from monomolecular films that modify wave characteristics and hence reflectance properties of surfaces. However, the lipid materials postulated to form monomolecular layers constitute only a small fraction of the organic material in either bulk seawater or at the surface 4-7, may not retain their strong surface activity in the presence of natural seawater dissolved organic material (DOM)8, and have not been found in any consistent relationship with slicks^{7,9-11}. The apparent association of slicks with macroalgae has also been noted¹²⁻¹⁵, but there has not been a demonstration of macroalgae-derived DOM in slicks. I show here that enrichments of UV-absorbing phenolic materials, consistent components of surface microlayers16, offer reliable indication of slick conditions. I then suggest that: (1) slicks result not from monomolecular films, but from viscosity changes in surface microlayers caused by more soluble organic components; and (2) the occurrence of slicks therefore depends not on absolute concentrations of surface-active materials, but on relative viscosity differences. Evidence is also given demonstrating the presence of macroalgae-derived phenolic materials in slicks.

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Surface microlavers were collected from Maine estuarine and coastal waters and from the North Atlantic ocean by the glass plate method^{17,18}. Microlayer samples and appropriate reference bulkwater samples were each filtered through precombusted, rinsed glass fibre filters. The filtrates were assayed for phenolic materials using UV absorbance at 280 nm in 10 cm quartz cells and a modification of the Folin-Ciocalteu test and for DOC by persulphate oxidation and IR detection^{20,21}. Microlayer enrichments were calculated as the ratio between the concentration of a substance in microlayer and in bulkwater samples. Particular attention was given to surface conditions at the time of sampling. The following classification system was used: (1) certified slicks—visually obvious slicks with at least some distinct boundaries; (2) slick influenced samples—conditions in which, although distinct boundaries were absent, the surface had some combination of stable bubbles, particulate debris, or viscous appearance during sampling which was characteristic of certified slicks; (3) mixed samples—samples in which some dips of the plate were in areas designated as certified slick or slick influenced surfaces and some were in clean areas;

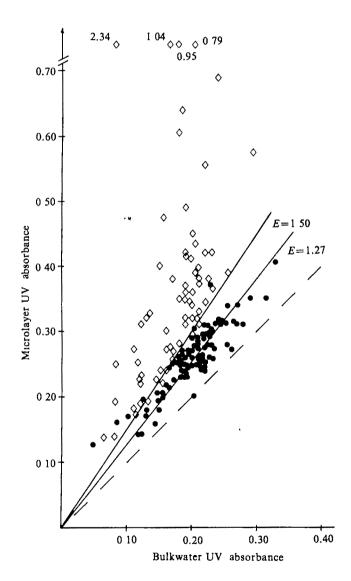


Fig. 1 Microlayer phenolic content versus bulkwater phenolic content, as measured by absorbance at 280 nm. Data were collected May 1978 to August 1981 from estuarine, Gulf of Maine, and North Atlantic (39° N, 66° W) waters. Lowest line indicates equal absorbance in microlayer and bulkwater samples. Line with slope of 1.27 $(r^2 = 0.99)$ denotes mean phenolic enrichment of clean microlayer samples. Line showing enrichment of 1.50 represents boundary between clean and slicked microlayers. •, Clean surfaces; \diamondsuit , slicked surfaces.

Ų,

Table 1 Slick and clean enrichments for phenolic materials and DOC DOC enrichment Phenolic enrichment Type Range* Range Ŧ 30 2.49 1.52-6.12† 1.39 0.79-1.79† Certified (0.192-1.04) 1.52-28.54 (2.92 - 11.87)1.78 0.79-4.92 31 3.33 (2.92-18.10)(0.192 - 2.34)41 2.39 1.65-3 57 1.41-2.47 0.89-4.68 Influenced 24 1.69 (0 137-0.421) (3.65-11.19)Mixed 13 2.00 1 48-3.06 0.81 0.58-1.03 (2 15-9.46) (0.172-0.555)

0.99-1.88

(0.125-0 405)

41

5‡

1.13

0.54-1.54

(1.91 - 3.92)

(1.82 - 12.32)

97 1.27

Clean

and (4) clean surfaces—no slicks, no visible accumulations of debris, stable bubbles rare or absent.

The slick data are summarized in Table 1, with clean surface data included for comparison. Only the phenolic materials showed a consistent relationship to slick conditions. All three slick classifications had mean phenolic enrichments greater than did clean surfaces. The mean phenolic enrichment in certified slick samples was significantly greater (P < 0.05) than the mean enrichment in slick influenced samples, with the mixed sample enrichments intermediate to and not significantly different from either the certified or influenced samples. In contrast to the phenolic materials, slick DOC enrichments were not significantly different (P > 0.05) from clean enrichments. Although the highest DOC concentration was in a dense slick, high DOC concentrations were not consistently related to the occurrence of slicks. DOC data from Pacific coastal and oceanic waters were similarly non-representative of the presence of slicks14. DOC as measured undoubtedly includes, in addition to relatively insoluble materials contributing to surface enrichments, varying quantities of more soluble materials. Therefore, it is not surprising that neither enrichments nor concentrations of DOC were reliable indicators of slick conditions. Phenolic materials are, however, preferentially enriched in microlayers relative to DOC¹⁶, and they represent the first fraction of DOC which consistently indicate slick conditions.

The slicks were distinguished by enrichment of phenolic materials rather than by concentration (Fig. 1). Slicks were evident throughout the UV absorbance ranges of both subsurface and microlayer samples, indicating that no minimum amount of phenolic materials was necessary for slicks to appear. Instead, the line representing an enrichment ratio of 1.50 clearly distinguished slicked from clean surfaces. This observation, that slicks were indicated by relative (enrichment) rather than absolute (concentration) measures, independent of the type of material measured, is inconsistent with the model of wavedamping by an incompressible film. In that model, wavedamping requires some critical concentration of surface-active molecules, independent of bulkwater concentration, above which slicks should ideally always be evident and below which no slicks should occur. The phenolic evidence indicates a relative relationship-slicks becoming evident when the UV absorbance in the microlayer reaches a ratio of 1.50 relative to the UV absorbance in bulkwater, compared with an average clean surface ratio of 1.27. I propose that the relative nature of the slicks observed here, indicated by phenolic materials, is due to relative differences in microlayer viscosity.

In organic-free seawater, damping of capillary waves at a clean interface is related to the viscosity of the seawater²². Organic materials in natural seawater will alter the viscosity of both the bulk seawater and, if they accumulate near the surface,

^{*} Range is range of enrichments; ranges of absolute values, in units of absorbance at 280 nm and mg 1^{-1} DOC, respectively, are in parentheses.

[†] Data set excludes extreme values from a dense slick so as not to bias comparisons with other categories. Extreme value is included in next data set. ‡ Data included from ref. 14.

of the surface microlayer where the viscosity changes may be enhanced by interfacial effects. When the amount of organic material in a microlayer increases the viscosity relative to bulk seawater and to adjacent but less enriched microlayers so that wave characteristics are altered, slicks will become evident. Slicks thus become a function of the viscosity in surface microlayers rather than of the compressibility of a monomolecular film, and the appearance of slicks depends on relative viscosity differences between slicked and clean microlayers, both relative

One implication of a relative viscosity hypothesis is that at a constant bulkwater viscosity, a microlayer viscosity gradient should exist between fully clean and fully slicked surfaces. In confirmation of this, the slick influenced samples did have a mean phenolic enrichment intermediate to clean and certified slick samples. In addition, in situ measurements of 'film pressures', which are generally performed without definitive demonstration of the presence of surfactant monolayers, probably represent an interaction not between the spreading oils

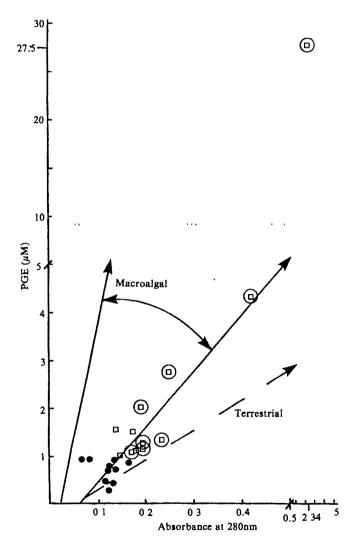


Fig. 2 Absorbance at 280 nm plotted versus phenolic reactivity expressed as micromoles of phloroglucinol equivalent (PGE). Dashed line indicates ratios typical of terrestrially-derived phenolic materials, while solid lines outline ratios of macroalgal-derived materials . Sources of bulkwater (), clean microlayer (□) and slicked microlayer (□) materials with absorbances <0.20</p> cannot generally be distinguished. However, in some slicks with higher absorbances and reactivities, ratios indicated macroalgaederived material. Samples collected October 1979, August 1980, and August 1981 in coastal waters of the Gulf of Maine, 0-30 km offshore. Salinity ranges: 29.5-33%. Scales change at 0.5 absorbance and at 5 µM PGE.

and a monolecular film but between the spreading oils and the natural microlayer organic materials. From this viewpoint, there is some evidence of a gradient from clean surfaces to weak and then sharply defined slicks²³ which supports the suggestion of the relative nature of slicks.

A second implication is that as the concentration of DOM in both clean and slicked microlayers increases in response to increased bulkwater DOM, the viscosity will also increase. Although the appearance of a slick in one area will depend on viscosity differences relative to clean surfaces in that area, both the slick and clean surfaces should have greater viscosities and different capillary wave characteristics relative to slick and clean surfaces in an area of lower bulkwater DOM. For example, in the low DOM North Atlantic samples, visual distinction between slicks and clean surfaces was less certain than in coastal waters, perhaps because of lower surface DOM concentrations and lower viscosities. It is possible that such differences between areas of different DOM, for both clean and slicked surfaces, might be detectable and quantifiable by remote sensing instruments, and alternatively, might affect measurements by such instruments.

It remains then to consider the UV-absorbing phenolic materials as constituents of slicks, and their sources. The IR evidence of Baier²⁴ suggesting highly hydroxylated components in natural slicks could apply to phenolic materials. A polymeric nature has been suggested for terrestrial and macroalgal materials showing phenolic characteristics^{25,26} and the phenolic materials might be expected to interact with amino acids, car-bohydrates, or lipid materials²⁷⁻³⁰ to form complexes similar to hypothesized microlayer compounds 7,24,30. The complex chemistry of the phenolic materials in seawater precludes assigning DOC values to their UV absorbance (refs 31, 32 and D.J.C., L. M. Mayer and M. L. Brann, unpublished data). There are, however, indications that microlayer materials and substances extracted from fucoid macroalgae can increase the solution viscosity of seawater³³, although it remains difficult to extrapolate that information to viscosities in microlayers.

It has been shown that in low UV absorbance, low phenolic content offshore and oceanic waters, it is difficult in both bulkwater and in microlayers to distinguish terrestrial and macroalgal sources for the phenolic materials¹⁶. In slicks, however, where the amounts of UV-absorbing materials can be large, there was definite evidence that macroalgae-derived phenolic materials contributed to slick DOM (Fig. 2). In Maine coastal waters, macroalgae have a seasonal exudation pattern³² which, perhaps accompanied by seasonal changes in reactivity of the exuded phenolics (D.J.C., L. M. Mayer and M. L. Brann, unpublished data), may contribute to the seasonal occurrence patterns of slicks11

The above observations do not preclude other compounds, such as lipids, as components of slicks, or negate physical slick formation processes, such as convergences. However, whatever the formation processes and regardless of additional components, enrichments of UV-absorbing phenolic materials provided consistent indications of slick conditions in all coastal waters sampled. It is possible that phenolic materials may be ubiquitous components of clean and slicked surface microlayers and might serve as indicators of slicks in other areas, especially coastal areas, of the oceans.

I thank the faculty, staff, and students of the Department of Oceanography, University of Maine, for assistance and encouragement. Supported by NSF: OCE-79-20244.

Received 13 October 1981, accepted 9 February 1982

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Lead 210 and moss-increment dating of two Finnish Sphagnum hummocks

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Peat materials are economically and ecologically valuable and so their dating is important. The lead isotope 210Pb $(22.26\pm0.22 \text{ yr})$ has had great value in dating recent deposits1-8 of the past 150 yr. But the varying conditions in different water reservoirs, and the possibility that the mathematical models used in ²¹⁰Pb-dating will give different results, call for additional chronological data. ¹⁴C-dating has major difficulties in this respect⁸⁻¹⁰ and dating with ¹³⁷Cs and ²³⁹Pu is not reliable ¹⁰⁻¹³. Previously ²¹⁰Pb-dating has been tried on only seven peat cores from ombrotrophic mosses: four from Denmark^{14,15} and three from England and Ireland¹⁰. We present here a comparison of ²¹⁰Pb results with moss-increment dates of selected peat material from Finland.

Two Sphagnum fuscum hummock cores from two Finnish peatlands, Kärpänsuo bog, Kuhmoinen (61°35' N, 25°20' E), core 1, and Kunonniemensuo bog, Kitte (62°05' N, 30°10' E), core F9, were used in this study. These cores had been previously dated by the moss-increment method^{11,16}. Both sampling sites are small, ombrotrophic, raised bogs with scattered small pines (Pinus silvestris L.) and derive their water supply exclusively from precipitation. The average water table in both hummocks is ~30-35 cm below the peat surface. The average peat depth at the sites is ~4 m. Most of the peat moss layers had grown in ombrotrophic conditions (according to pollen analysis these layers are younger than 6,000 yr BP), while the bottom layers of 50 cm or so were formed in minerotrophic conditions. The hummocks from which the cores were taken are several metres in diameter. They consist of a very loose S. fuscum carpet with dwarf shrubs such as Empetrum nigrum. Some Polytrichum strictum was found at both sites and the annual increments of this species served as a dating tool. The cores were taken with the 12-cm diameter steel corer and cut into sections, 2.5-5.0 cm thick. The peat material was slightly decomposed white moss (Sphagnum)11,16

The shape and microstructure of the individual mosses were used to identify the annual increments. These could be distinguished in Sphagnum by means of cyclic pigmentation¹⁷ branching pattern and changes in growth direction caused by pressure from the snow cover 11. About 15-20 stems of Sphagnum mosses and/or P. strictum vertically penetrating the peat sections were measured to determine their actual lengths after straightening. From these stems, the annual increments (which include 1 yr of growth) were identified 19 and their lengths measured. In the case of very short increments a stereomicroscope (×150) was also used to observe the changes in the stem pigmentation. The average number of years within each section was calculated by dividing the mean stem length with the mean increment length, so the values used represent an age interval in moss-increment years. Because the averages of the age intervals were not statistically weighted, no statistical distribution of the moss-increment years in the age intervals were given 11,16

Materials in the same peat samples were analysed for their ²¹⁰Pb contents at the Institute of Physics, Uppsala, Sweden and the Technical Research Centre of Finland, Espoo, Finland. The samples from the Kärpänsuo bog, core 1, contained only peat moss (S. fuscum) materials and all the visible high (vascular) plant remains were excluded. The samples from Kunonniemensuo bog, core F9, comprised the entire peat material. However, the percentages of the vascular plants were low in

all but the deeper subsamples.

The 'total' ²¹⁰Pb was measured in the two laboratories by determining the granddaughter product ²¹⁰Po by means of the isotope dilution. The ²⁰⁸Po was used as an internal yield tracer in both laboratories. These tracers were calibrated against different standards. The chemical separation of polonium samples in the two laboratories is described elsewhere 6,20,2 The samples were counted by α spectrometry using silicon surface barrier detectors. The long-term stability of these detectors was considered22. The radon emanation was used in Uppsala to determine ²²⁶Ra (ref. 23). The ²²⁶Ra was separated from the sediment residues, with the help of Ba-carrier, and kept in closed bottles for a month. A nitrogen gas was then used to flush the ²²²Rn to an ionization chamber that was carefully examined for impurity and adsorption effects^{6,20} in

The total ²¹⁰Pb of the peat samples comprises ²¹⁰Pb of atmospheric origin; unsupported ²¹⁰Pb and an additional *in situ* part which is produced from ²²⁶Ra; and supported ²¹⁰Pb. The supported ²¹⁰Pb may not always be in secular equilibrium with its precursor ²²⁶Ra. Supported ²¹⁰Pb was estimated at Espoo only from the total ²¹⁰Pb of samples older than 200 moss-increment years. The unsupported ²¹⁰Pb for such samples should be <2%. In Uppsala the ²²⁶Ra $(t_{1/2} = 1,620 \text{ yr})$ was also used to evaluate the supported ²¹⁰Pb. The ²²⁶Ra values of five samples, three of them being taken from one core, are in good agreement and the average value is 0.12 ± 0.04 pCi g⁻¹. This value agreed with the total ²¹⁰Pb values in the deeper samples showing 200-500 moss-increment years; the averages of the two laboratories are 0.07 ± 0.01 pCi g⁻¹ and 0.19 ± 0.03 pCi g⁻¹ for core F9 and core 1 respectively.

²¹⁰Po $(t_{1/2} = 138 \text{ days})$ is assumed to be in equilibrium with ²¹⁰Pb $(t_{1/2} = 22.26 \pm 0.22 \text{ yr})$ in all but the surface slice (²¹⁰Po reaches secular equilibrium with ²¹⁰Pb in about 2 yr). This assumption is valid only in ideal cases of closed systems in which neither the ²¹⁰Pb nor the ²¹⁰Po is subject to any kind of translocation in the time interval of interest. This assumption was confirmed in a sediment core²⁴. Lewis²⁵ estimated a mean residence time for metals similar to ²¹⁰Pb in organic-rich soils to be about 2,000 yr. Mosses of ages younger than 700 yr showed that 95–99% of the total lead is either bound or sorbed to the peat materials. Jacobsen 15 found that selected suboceanic ombrotrophic peats from Denmark have a very high capacity for retaining metals and could be used to give good records on atmospheric deposition, especially for lead 'petrol lead'. Pakarinen and Tolonen²⁶ claimed that mobility of lead might

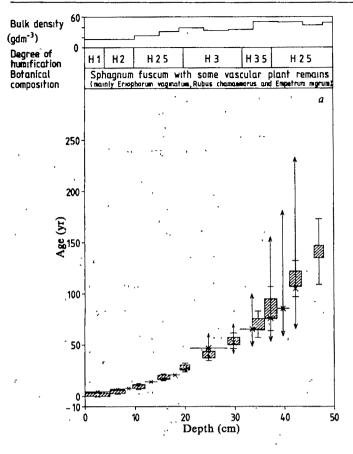
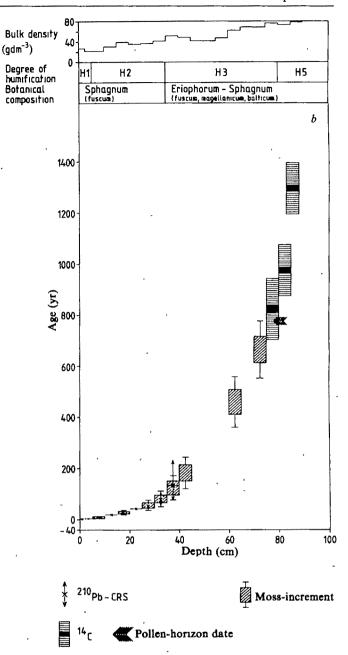


Fig. 1 Ages-depth profiles of peat layers from two Finnish Sphagnum-hummocks: a, Kärpansuo bog, core 1; b, Kunonniemensuo bog, core F9. Ages, in moss-increment years, are before 1975. Vertical bars represent, $\pm 1\sigma$, of the ²¹⁰Pb ages (Uppsala results) or average error for the moss-increment years. The lengths of the rectangles represent either the moss-increment age intervals or the uncertainties in the ¹⁴C ages.

affect the chronological order of the atmospheric lead in peat and give rise to enriched horizons.

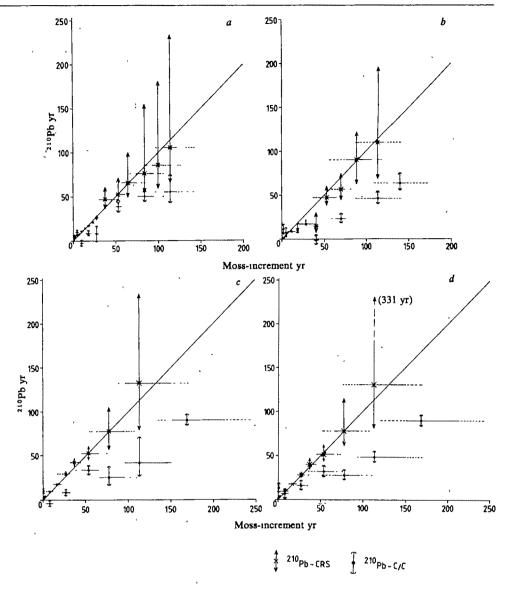
The ages of the peat layers from the Kärpänsuo bog, core 1, and the Kunonniemensuo bog, core F9, are given in Fig. 1: in moss-increment years these are before 1975 and their errors were based on direct observations. The ²¹⁰Pb-concentrations (per unit dry weight) which were measured at Uppsala and Espoo, using different chemical extractions and independently calibrated tracers, allow tracing of systematic errors in the laboratories and lend credibility to the evaluation of the ²¹⁰Pb models. The uncertainties in the ²¹⁰Pb values included not only the statistical uncertainties in the 210Po activity measurements but also those due to the 208Po tracers, the corrections of the polonium peaks for the silicon-detector instabilities (performed only in Uppsala) and the supported ²¹⁰Pb activity. For ²²⁶Ra, the uncertainties included the counting statistics, the background of the ionization chamber and the efficiency of the filling system as determined by a standard sample. The ages in ²¹⁰Pb yr were calculated using the constant initial concentration (CIC) and the constant rate of supply (CRS) models^{27,28}. The ²¹⁰Pb concentrations of the past 20–30 moss-increment years (the upper 15-20 cm) were normalized for their radioactive decay and a σ -weighted average was calculated for each core and laboratory. These averages served as initial concentrations for the studied cores (CIC model), and smoothed down horizontal variations of bulk densities and growth rates which took place in the surface peat layers. Such variations may cause the 210Pb concentrations to differ from place to place even if the atmospheric supply rate of the 210Pb was constant. These values, as calculated with aid of the least



squares method, were: for core 1, Kärpänsuo bog $5.50\pm0.22~\mathrm{pCi}~\mathrm{g}^{-1}$ for Uppsala and $4.70\pm0.35~\mathrm{pCi}~\mathrm{g}^{-1}$ for Espoo; for core F9, Kunonniemensuo bog, the Uppsala value was $5.43\pm0.27~\mathrm{pCi}~\mathrm{g}^{-1}$ and for Espoo $5.99\pm0.44~\mathrm{pCi}~\mathrm{g}^{-1}$. For the CRS model the integrated activities over the past 150 yr were: for the Kärpänsuo core, $33.3\pm3.0~\mathrm{nCi}~\mathrm{m}^{-2}$ and $44.8\pm3.6~\mathrm{nCi}~\mathrm{m}^{-2}$ for Uppsala and Espoo respectively; the corresponding values were $49.2\pm3.3~\mathrm{nCi}~\mathrm{m}^{-2}$ and $47.3\pm2.9~\mathrm{nCi}~\mathrm{m}^{-2}$ for the Kunonniemensuo core.

Figure 1 shows the age-depth relation for both cores. The 210 Pb ages, as calculated using the CRS model, show good agreement with the moss-increment dates. The radiocarbon dates for some deeper layers from core F9, Kunonniemensuo bog, are also given: Helsinki-1140, 75–80 cm, 800 ± 120 yr BP; Helsinki-1053, 80-85 cm, 950 ± 100 yr BP; and Helsinki-1141, 83-88 cm, $1,270\pm100$ yr BP. The dates were derived using Libby's half life (5,570 yr). The samples were used without further physical pretreatment and without the NaOH-insoluble fraction being dated. Unfortunately no δ^{13} C values are available to correct for the isotopic fractionation but if the suggested values for peat materials which range from -19% to -35% are used, an age correction by 95 to -145 yr could be expected.

Fig. 2 A comparison between the ²¹⁰Pb years as calculated using the CIC and CRS models and the moss-increment years of the Kärpansuo bog, core 1: a, Uppsala results; b, Espoo results and the Kunonniemensuo bog, core F9; c, Uppsala results; d, Espoo results. Vertical bars represent standard deviation, $\pm 1\sigma$, of the ²¹⁰Pb ages; the horizontal bars give the age intervals of the peat layers with their average errors in broken bars.



Thus, the 14C dates show good agreement with the mossincrement dating and the distinct onset of rye cultivation at 80 cm, which according to pollen studies by Vuorinen on an adjacent varved lake (Hinnisenlampi), occurred ~AD 1200. The bulk density of the top moss layers is lower than the underlying layers because of either or both compaction and decomposition of the peat materials. For both cores the CIC model underestimates age compared with the moss-increment method (Fig. 2) which suggest higher initial concentrations of the 'unsupported' ²¹⁰Pb in the deeper moss layers. This coincides with the calculations made on the annually accumulated dry matter which showed lesser net accumulation for the earlier peat layers 11,16. The CRS model shows a good agreement with the moss-increment method (Fig. 2). All the dates agreed within $\pm 1\sigma$ except for three dates from Espoo (the Kärpänsuo core) which deviated by -2σ . Note that the CRS model gives rise to higher statistical uncertainties in the final ages. As one moss-increment year represents a growing period which corresponds quantitatively to one calendar year, it seems likely that the atmospheric rate of the ²¹⁰Pb supply during the past 150 yr was constant, the annual peat layers of the cores effectively accumulated the available ²¹⁰Pb during their growth and the peat material behaved as a 'closed' system.

The main part of this study was supported by the Swedish Natural Science Research Council, the Natural Science Research Council of the Finnish Academy and the Finnish Foundation for Environmental Research Maj ja Tor Nesslingin Säätiö.

Received 22 October 1981, accepted 28 January 1982

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Seasonality and mean annual sea surface temperatures from isotopic and sclerochronological records

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Seasonality is one of the most dramatic climatic variables affecting the physical and biological properties of the surface of oceans. Attempts to deduce the seasonal temperature contrast of past oceans using the fossil record have led investigators to explore the carbonate growth patterns in certain marine invertebrate organisms. In many cases these growth patterns represent annual growth increments analogous to annual rings in trees1. Just as tree rings contain a record of terrestrial climates2, systematic variations in the physical or chemical properties of annual growth increments in the skeletons of marine organisms record the environmental or climatic properties of the marine realm. Several investigations have called attention to the use of stable isotope profiles in growth increments of mollusc shells to determine the seasonal water temperature changes³⁻ seasonal timing of coastal upwelling8. Mollusc shells therefore have the potential of revealing much information about past environmental changes9, particularly along temperate continental shelves where our knowledge of the palaeoceanographic history is very limited. We have now used coordinated isotopic and sclerochronological (growth increment) studies to compare the geochemical history of shell growth in Spisula solidissima with the physical oceanographic history of the Middle Atlantic Bight and determine whether the isotopic records of S. solidissima from different water depths on the continental shelf reflected the seasonally different thermal regimes at those depths. We chose S. solidissima because it is widely distributed along temperate continental shelves, its shell growth and life history are well understood^{9,10}, and the genus has a fairly wide distribution in Plio-Pleistocene fossil deposits. Jones 11,12 has already established through a detailed sclerochronological analysis of S. solidissima that a 10-20 yr time series of mean sea surface temperature trends can be resolved from the record of annual shell growth. A strong negative correlation exists between growth increment size and yearly mean water temperature.

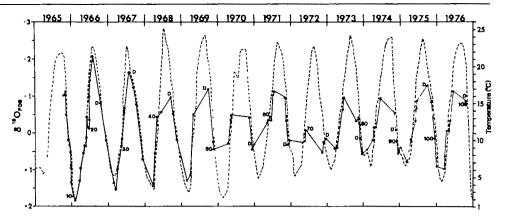
Live specimens of S. solidissima were collected from two localities off coastal New Jersey in the Middle Atlantic Bight, from water depths of 10 and 45 m. Growth increment analyses were performed whereby yearly changes in shell growth height were corrected for ontogenetic growth trends^{11,12} by dividing the measured shell height by the expected growth predicted using the Van Bertalanffy curve as described by Caddy and Billard¹³. This treatment yields a standardized index that can be compared among individuals of any size (age)¹¹. Between 7 and 23 isotopic samples of aragonite powder were secured from each annual growth band by microdrilling or filing of the outer shell layer. The inner layer was avoided to eliminate potential isotopic effects due to shell dissolution and reprecipitation. Annual dark growth increments in the outer layer provide reference points to differentiate successive years' of growth and to prevent sample overlap¹¹. Stable isotopic analyses were performed according to established procedures14 (all isotopic data are given in δ notation as the % enrichment or depletion relative

to the PDB carbonate standard¹⁵). A total of 150 separate isotopic analyses were made for an 11-yr period (1965-76) from the inshore 10 m specimen (INS no. 1), and for the calendar year 1966 in two offshore specimens from 45 m water depth (OFF nos 3, 5). The stable isotopic data sets for the calendar year 1966 were compared with surface and bottom temperature and salinity data available for the collection area^{11,16,17}. The exact year of growth could be determined if the collection date was known and the sclerochronological analysis of each shell^{9,11} performed.

The 11-yr isotopic time series from the 10-m site exhibits a regular periodicity and confirms that the δ^{18} O of the outer shell layer of INS no. 1 monitors the record of changing seasonal water temperature but not in a linear fashion (Fig. 1). The yearly isotopic amplitude (Δ^{18} O) decreases steadily from 1965 to 1972 and increases again from 1972 to 1976. The Δ^{18} O values have a maximum range of 3.8% during the first year of growth (1966). A straightforward interpretation of the isotopic range in terms of seasonal contrast would suggest wrongly that a steady decrease in seasonal contrast occurred from 1965 to 1972, with the seasonal amplitude increasing in years 1973-77. This interpretation is not supported by historical oceanographic data for the Middle Atlantic Bight 16,17. Shell growth in S. solidissima is apparently inhibited, but not totally stopped, during years with unusually warm mean sea surface temperatures (MSST) (>12.5 °C), and the observed yearly Δ^{18} O is reduced. During 1972, for example, when MSST was 13 °C, the Δ^{18} O values would suggest a seasonal contrast of <5 °C and unusually warm winter minimum and cold summer maximum temperatures (Fig. 1). Sclerochronological analysis of INS no. 1 revealed that the addition of shell height was maximum during the first year of growth and decreased almost exponentially until the eighth year of growth when a slight increase occurred again. Jones¹¹ had previously demonstrated that the size of annual growth increments in populations of S. solidissima are highly correlated with yearly mean sea surface temperatures. Shell growth conditions are apparently most favourable during years which have generally cooler water temperatures (MSST < 12.5 °C). A plot of the yearly isotopic amplitude (Δ^{18} O) versus a standardized growth index and mean sea surface temperatures (Fig. 2) reveals a similar relationship. The yearly isotopic range (Δ^{18} O) is negatively correlated (r =-0.77) with MSST and shows a strong positive correlation with the growth index (r = 0.79). Δ^{18} O values are therefore large and closer to the annual isotopic amplitude expected from the seasonal temperature contrast when MSST conditions are favourable to growth (Fig. 1). Periods of abnormally low growth thus best explain the diminished Δ^{18} O values. The Δ^{18} O record is, therefore, best interpreted as a function of MSST, and not as a measure of the annual temperature contrast or seasonality except possibly in optimum years of growth. The significant correlations in Fig. 2 suggest that the $\Delta^{18}{\rm O}$ values may be used to estimate the MSST of the Middle Atlantic Bight to within better than 0.5 °C.

It is desirable, therefore, that growth increment analyses be done concomitantly with detailed stable isotopic analyses of molluscs to interpret seasonality information from mollusc growth records properly. Even though the δ^{18} O at the outer shell margin of a particular mollusc may seem to reflect the approximate season that a specimen is collected, the isotopic amplitude cannot be strictly interpreted in terms of seasonality. The Δ^{18} O in particular species must be interpreted in the context of the growth history of the particular mollusc as was emphasized as early as 1953 (ref. 3). In the use of S. solidissima, we are fortunate that calibration of the Δ^{18} O, growth increments and seasonality can be made before investigation of fossil specimens. Once this calibration is made, it should be possible to decipher quantitatively important properties of continental shelf waters using isotopic and sclerochronological studies of fossil specimens. Although it is not possible to use S. solidissima to obtain a time series of seasonality, the mollusc may have the potential for recording the seasonal temperature amplitude on

Fig. 1 Oxygen isotope signal (solid line) for 11 yr of growth in a specimen of Spisula solidissima (INS no. 1) collected alive from a depth of 10 m on the Mid-Atlantic Bight off New Jersey. The dashed line corresponds to the monthly mean sea surface temperature for 1965-76 at the collection site. Isotopic data within a given year can be plotted knowing the growth rate of the specimen and the approximate position of the 'dark' growth increment which is laid down during the late summerearly autumn months 11,12,18.



the continental shelf. A test of this can be seen in Fig. 3 where the δ^{18} O signal for the year 1966 from INS no. 1 is plotted relative to the record of sea surface temperature (SST). The δ^{18} O data yield two types of information: (1) that the magnitude of the seasonal temperature contrast was accurately tracked; (2) that shell growth was continuous but varied systematically throughout the year in the following manner. Growth rates were most rapid during late spring-early summer (March-June).

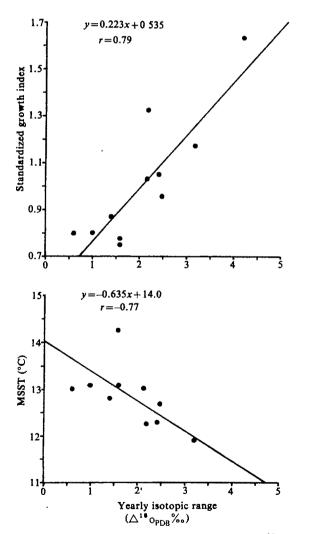


Fig. 2 The relationships of the yearly isotopic range (Δ^{18} O %) with the standardized growth index 11,12 and yearly mean sea surface temperatures (MSST) for 11 yr of growth in S. solidissima (INS no. 1). The best fit lines from linear regression analysis are also shown. The standardized growth index is computed by dividing the measured shell height by the expected growth using the Van Bertalanffy growth curve $^{11-13}$.

·d.

In July, growth slowed but decreased to a minimum during the SST maxima in August through October, when spawning and deposition of the characteristic dark increment occurred. Shell growth then increased again as SST cooled in October through December. This confirms the annual cycle in shell growth that has been quantitatively defined through biweekly observations of living S. solidissima populations from this region of the continental shelf ^{12,18}.

To determine whether these results could be used to differentiate the thermal structure on the continental shelf off New Jersey, the detailed δ^{18} O record for the year 1966 in INS no. 1 (10 m) was compared with that of two specimens collected live from 45 m water depth (OFF nos 3, 5) (Fig. 3). During January to April-May, the 10 m and 45 m specimens have identical δ^{18} O signals. From May to September, the 10 m and 45 m records clearly diverge as the surface waters continue to warm and the temperature gradient increases to a maximum in August. As the surface and 45 m water temperatures begin to converge in October to January, so do the δ^{18} O values of the inshore and offshore specimens. These trends lead us to believe that the isotopic records of these specimens are accurately monitoring seasonal contrasts in the surface waters as well as the seasonal development and breakdown of the thermo-cline between the inner and middle shelf¹⁹. As fossil specimens of S. solidissima which grew at different depths may be differentiated on the basis of their shell morphology and growth curves¹², it may be possible to reconstruct the thermal stratification along the continental shelf from fossil shells.

A detailed sclerochronological and stable isotopic study of annual growth increments in S. solidissima collected live has enabled us to establish confidence limits about the use of mollusc isotopic data in palaeoseasonality studies. Sclerochronological analysis enables optimum years of shell growth to be identified. Although the annual isotopic signal (Δ^{18} O) is qualitatively recorded in all years of growth, the magnitude of the Δ^{18} O in all years is not linearly related to the seasonal sea-surface temperatures. The first years of growth in S. solidissima seem to yield the best potential for quantitatively determining the seasonal contrast. It is possible, therefore, to use the isotopic data in S. solidissima to monitor successfully the development of the thermocline between the inner (10 m) and middle (45 m) continental shelf when the growth histories of the mollusc shells are understood. The carbon isotopic record of S. solidissima may also be utilized to decipher seasonal productivity and/or water mass stratification²⁰. Thus, calibration of the isotopic signals of molluscs like Spisula within a physical and biological framework may provide a powerful means to reconstruct the palaeo-oceanographic history of temperate continental shelves, especially during the Plio-Pleistocene for which numerous fossil deposits are available. Such valuable palaeoclimatic information has hitherto been unavailable from temperate continental shelf areas, and only possible where corals are present. Due to the thermal tolerances of mollusc species, however, caution may be required in palaeoseasonality studies of other mollusc species.

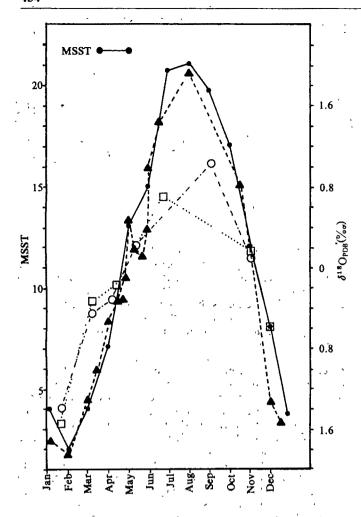


Fig. 3. A comparison of the seasonal change in monthly MSST for 1966 and the oxygen isotopic composition (δ^{18} O %) of the same year of growth in Spisula from water depths of 10 m (INS no. 1 ▲) and 45 m (OFF nos 3 O; 5 □). The isotopic data are plotted knowing the growth rates of the individual specimens and the position of the 'dark' growth increment which is laid down during the late summer-early autumn months 11,12,18.

The analytical work of this research was supported by the NSF. This is contribution no. 441 of the Belle W. Baruch Institute for Marine Biology and Coastal Research of the University of South Carolina. We thank Patricia Kelly and Kitty Harper for typing the manuscript.

Received 5 August 1981; accepted 21 January 1982

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Sedimentary record of rainfall variations in a sub-humid lake

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The physical, chemical and fossil characteristics of lake sediments are frequently used to study environmental processes and change within a lake, in the lake catchment and over a wider area. Analysis of sediment dry weight variations in a 1.3-m core from a lake, Dayat Afougach, in Morocco suggests that they record both seasonal and annual rainfall variations and the associated catchment erosion and lake sedimentation processes. If this is a common feature of certain lake sediments then these lakes may prove to be a source of information about changing precipitation patterns in areas where climatological records are not available, as well as providing information on changing sediment yields from their catchments.

Dayat Afougach (31°21' N, 5°07' W) is a 32 hectare eutrophic lake situated at an altitude of ~1,500 m in the Middle Atlas Mountains. The catchment area of the lake is roughly 1,400 ha and it is underlain by Mesozoic calcareous rocks1. The mean depth of the lake is around 6 m and it has an outflow but no perennial inflow. The lake is in a semi-arid region², but it has a fairly high annual rainfall (mean for 1927-49) of 1,100 mm (ref. 1) due to its high altitude. The climate corresponds to the Mediterranean winter-wet summer-dry sub-humid climate type (see Fig. 4a). A basic limnological description of the lake has been made by Gayrals3.

An undisturbed sediment core4 was taken at the deepest part of the lake in the south-east from 11 m on the 25 September 1979. There was no visible stratification of the calcareous sediment. It was sectioned at 1 or 2 cm intervals and the sediment dry weight found after drying to 105 °C. The sediment was rendered soluble by HF-HClO4 digestion⁵ and organic content estimated by loss on ignition⁶. Metal concentrations in the digests were determined by atomic absorption spectrophotometry, phosphorus colorimetrically and the precision of the results estimated by duplicates. The standard deviations for seven duplicates are 16 mg Ca per g, 3 mg Mg per g, 0.3 mg Na per g, 0.3 mg Fe per g, 0.06 mg P per g, 5 µg Mn per g, 2 µg Cu per g, 6 µg Zn per g, 15 µg Pb per g and loss

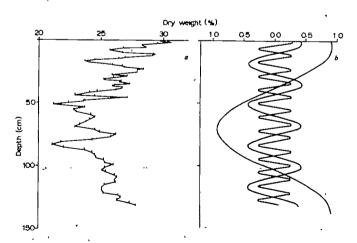


Fig. 1 a, The variation of sediment dry weight with depth in Dayat Afougach. b, The three Fourier harmonics, first, fourth and eleventh, which describe the dry weight behaviour in a The scale indicates how much each harmonic causes the dry weight to depart from its mean value of 25.4%.

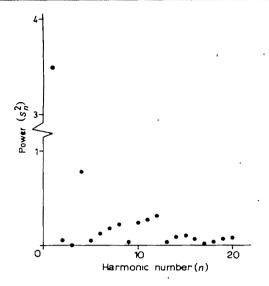


Fig. 2 The Fourier power spectrum of the dry weight results.

on ignition 1.3%. The dry weight results and annual rainfall totals from 1958 to 1979 for Ifrane, 23 km south-west of Afougach⁸, were subjected to Fourier analysis⁹.

The variation of sediment dry weight with depth (Fig. 1a) shows repetitive patterns and the Fourier power spectrum is shown in Fig. 2. The first harmonic accounts for 48.5% of the variance and has a wavelength of 131 cm, the length of the sequence. The sequence is too short to provide any evidence for a repeating unit of this wavelength. This harmonic only describes the general trend of increasing and decreasing values. An F-test shows the fourth harmonic accounts for a significant (0.005 < P < 0.01) amount of the variance (11.0%). It has a wavelength of 33 cm. The sixth, seventh and eighth harmonics, even taken as a group, are not significant (0.05 < P < 0.10). The tenth, eleventh and twelfth harmonics together make a significant contribution to the variance (0.005 < P < 0.01) and account for 11.9%. They have wavelengths of 13, 12 and 11 cm, respectively, and so with a wavelength around 12 cm account for the smallest scale repeating pattern in the dry weight results (Fig. 1a). Decomposed eleventh, fourth and first harmonics are shown in Fig. 1b. The contribution of the fourth harmonic is best seen when it is in phase with the eleventh harmonic at the pronounced dry weight minima around 15, 50, 83 and 120-cm depth. Two lines of evidence indicate that these dry weight patterns reflect rainfall variations.

The first concerns the smallest scale pattern, harmonics 10/11/12. This has a wavelength around 12 cm and is probably caused by an annual sedimentation cycle. The sediment accumulation rate would then be around 12 cm yr⁻¹. Three pieces of evidence support this interpretation. First, Wilson¹ has shown that there is a strong relationship between monthly sediment yield and monthly rainfall in catchments with a Mediterranean type climate. Assuming that the sediment has a larger particle size during the periods of higher yield, then the variation in monthly rainfall over a year (see Fig. 4a) could cause the smallest scale dry weight pattern by controlling the grain size of the allogenic sedimentary inputs and hence the dry weight^{11,12}. During the wetter months, especially those following the dry season¹⁰, the allogenic inputs would be coarser than during the drier months and would produce the sediment layers of higher dry weight. Second, the relationships of organic content, sodium, magnesium and iron concentration to dry weight (Fig. 3) also indicate that the dry weight variations are due to particle size variations. Organic content and sodium and iron concentration are higher in clay-sized sediments 13-15 and magnesium is frequently higher in the silt fraction 15,16, so the dry weight axis in Fig. 3 corresponds to particle size. None of the other elements determined (Ca, P, Mn, Cu, Zn, Pb) show

a significant relationship with dry weight. That the calcium concentration is constant suggests that the seasonal precipitation of calcium carbonate is not responsible for the patterns observed. Third, the implied accumulation rate of around 12 cm⁻¹ is reasonable in this environmental setting. Around 1-22.5 cm yr⁻¹ have been found in reservoirs with varying catchment size in the drier areas of the USA^{17,18} and a rough sediment yield from the catchment of around 900 tonnes km⁻² yr⁻¹, calculated assuming the accumulation rate, a mean sediment wet density of 1.25 g cm⁻³, no losses through the outflow and even sedimentation, is also reasonable in this environmental setting. Sediment yield is very variable but

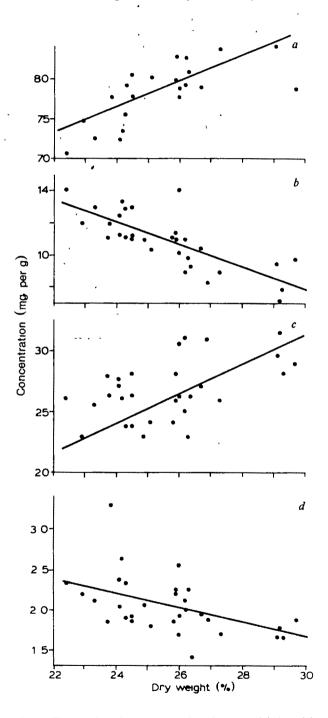
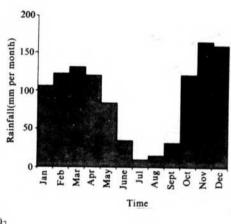
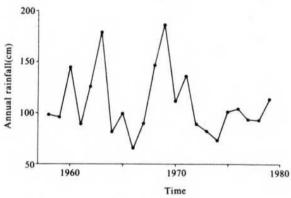


Fig. 3 The relationships between mineral content (a), iron (b), magnesium (c) and sodium (d) concentration and sediment dry weight in Dayat Afougach sediment. a: % mineral = 40.7 + 1.5 wt%, n = 23, $r^2 = 0.38$, 0.001 < P < 0.01. b: mg Fe per g = 28.8 - 0.698 wt%, n = 31, $r^2 = 0.57$, P < 0.001. c: mg Mg per g = 9.9 + 0.659 wt%, n = 31, $r^2 = 0.25$, 0.001 < P < 0.001. d: mg Na per g = 4.4 - 0.091 wt%, n = 31, $r^2 = 0.24$, 0.001 < P < 0.01.

around 600 and 700 tonnes km⁻² yr⁻¹ are suggested (ref. 19 and ref. 20 respectively) for this setting. The dry weight results in Fig. 1 show that, although the mean distance between peaks of the smallest scale pattern is around 12 cm, the distance varies from 8 to 18 cm. This behaviour appears to be accounted for by some of the harmonics on either side of 10/11/12, especially 7, 8 and 15, and may be due to annual variations in sediment yield from the catchment. Also, the dry weight-depth profile becomes damped and rounded below 100 cm. This may be due to compaction and to the 2-cm intervals used in sectioning the core at this depth. However, overall the three pieces of evidence suggest that the smallest scale repeating dry weight pattern reflects seasonal rainfall variations.

The second line of evidence suggests that the other two components of the dry weight variation reflect annual rainfall variations. Annual rainfall totals for Ifrane from 1958 to 1979 are shown in Fig. 4b and their Fourier power spectrum in Fig. 4c. The sequence is too short to provide reliable evidence that the third harmonic, with a wavelength around 7 yr, is a real environmental feature. This harmonic does, however, describe the decreasing rainfall in the early 1970s followed by a recovery, a pattern that was found over the whole of North Africa2 Assuming the 12 cm yr⁻¹ accumulation rate, then the low sedi-





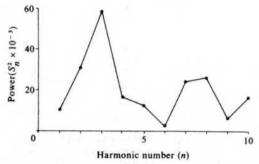


Fig. 4 a, The variation of monthly rainfall at Ifrane based on results from 1927 to 1949. b, The variation of annual rainfall at Ifrane from 1957 to 1979. c, The Fourier power spectrum of the annual rainfall at Ifrane from 1957 to 1979.

ment dry weight values between 50 and 90 cm, described by the first harmonic, correspond to the years 1971-74. Thus the general trend of decreasing and increasing sediment dry weight (Fig. 1a and first harmonic in Fig. 1b) reflects the trend of decreasing and increasing annual rainfall at Ifrane in the 1970s (Fig. 4b). The seventh and eighth harmonics in the Ifrane rainfall results have wavelengths of 2.9 and 2.6 yr. Having a wavelength <3 yr this component is reflected in the dry weight results by the fourth harmonic, which, assuming a 12 cm yr accumulation rate, corresponds to a wavelength of 2.8 yr. This, on average, just less than 3 yr rainfall cycle is probably part of the widespread quasi-biennial oscillation of many meteorological properties, including rainfall^{21,22}. The quasi-biennial oscillation has also been found in lake varves and tree rings23. That the fourth and eleventh dry weight harmonics are in phase (Fig. 1b) also supports the suggestion that the fourth harmonic corresponds to year to year variations.

The above evidence, although indirect, does provide a coherent picture of sedimentation in Dayat Afougach greatly influenced by rainfall variations. Establishing the accumulation rate directly by sediment dating and particle size analysis is, nevertheless, desirable to increase the reliability of the interpretation. Unavoidable loss of the core prevented particle size analysis being carried out. The alternate sediment layers of different chemical constitution and particle size in Afougach may be termed seasonal rhythmites and these have been found in other lakes^{24,25}, including semi-arid ones¹⁷. The seasonal rainfall variation at Afougach seems to provide the periodic variation in allogenic sedimentary input. Although annual variations in the flood events in another lake were found to be recorded in the sediment dry weight profile18, whether the calcareous nature of the Afougach sediment is important in allowing rainfall variations to be recorded is not known at

present. However, as the monthly sediment yield from catchments in areas with seasonal climates is dependent on rainfall10, sediment dry weight results may be a source of rainfall information in such areas, especially where there is little physical or biological mixing of the sediment.

I thank the other members of the group which visited Morocco in 1979, Roger Flower, Bob Nash, Alan Perrott and Doug Radford. Also, The New University of Ulster, The Royal Society, British Museum (Natural History), British Association for the Advancement of Science and the Royal Geographical Society for supporting the fieldwork and Ben Saied for his help in Morocco.

Received 26 October 1981; accepted 2 February 1982.

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Modern archaeomonads indicate sea-ice environments

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Diatoms, radiolarians, silicoflagellates, ebridians and archaeomonads are the primary groups of pelagic organisms in siliceous marine deposits, with only the archaemonads regarded as being absent from modern oceans and sediments. They remain a poorly known group of siliceous nanofossils in Cretaceous to Upper Tertiary marine sediments. We present here the first report, from any modern oceanic environment, of pelagic cysts that conform to the descriptions for Archaeomonadaceae. These cysts occurred in seawater, sea ice and marine-surface sediments, demonstrating the group is still extant. Furthermore, their distribution in modern sediment suggests they may be useful guide fossils for sea-ice environments as they occur abundantly only in polar or near-polar water where sea ice is present. Few other microorganisms seem to have such a close and exclusive association with sea ice, thus archaeomonads deserve increased attention as indicators of ice conditions for deep-water marine environments.

Small (7-20 µm) siliceous cysts found as minor constituents of many Upper Cretaceous to Pleistocene sediments, archaeomonads dominate few sedimentary assemblages^{1,2}, and thus their environmental affinities are poorly known. First described as a taxonomic group^{3,4}, archaeomonads are generally considered to represent resistant stages in the life histories of unknown or extinct marine chrysophytes, as they closely resemble the statocysts produced by freshwater chrysophytes⁵ Both archaeomonads and chrysophyte cysts are siliceous spheres possessing a single pore that is usually surrounded by a flange. Surfaces vary from smooth to spiny and are important taxonomic features in both taxa. Pelagic chrysophyte cysts have been reported from oceanographic surveys in the Weddell Sea10 and other areas11, but little or no taxonomic, descriptive or morphological information is given in these reports, making it impossible to determine whether the cysts belong to the Archaeomonadaceae or some other group such as those described by Silver et al. 12. Moreover, previous studies have relied on light microscopic examination of material, but careful descriptions of the diagnostic features of these nanoplanktonsized organisms require scanning electron microscopy (SEM).

Fifty-three water samples were obtained from a 10,000-km² area centred around 74°27′S, 37°53′W during the 1978 International Weddell Sea Oceanographic Expedition (IWSOE)¹³. The samples were taken at depths of 2–108 m; temperatures of –1.844 to –1.697 °C; oxygen concentrations of 6.85–8.79 ml per l, salinities of 34.01–34.94‰, and bottom depths of 377–1,982 m. All samples were taken at least 50 km from land or glacier in a water column averaging 500 m in depth. Water samples were filtered through Nuclepore filters (0.4 µm pores) and oven dried at 50 °C. This sample processing method destroys cellular components, making it impossible to study the vegetative cells associated with cyst hard parts, but leaves skeletal material clean and unharmed.

To prepare the filtered specimens for SEM, filters were coated with gold and examined using a JEOL JSM2 scanning electron microscope. To determine the wall composition, individual archaeomonads were analysed by X-ray fluorescence in a Cambridge S-180 SEM with an ETEC EDAX attachment. Counts were made across filters during routine SEM using transects of known area.

Twenty-three of the Weddell Sea water samples examined contained archaeomonads. Their average abundance was $1.4 \times 10^3 \, \mathrm{I^{-1}}$ in the seawater samples, (s.e. 2.8×10^2 , the maximum was $9.4 \times 10^3 \, \mathrm{I^{-1}}$). Archaeomonads were found in 20 out of 29 samples from 0 to 55 m and in 3 out of 24 samples from 55 to 110 m. Archaeomonads abundance decreased significantly with depth (P < 0.01). Four species were found in the Weddell Sea water column, Archaeomonas areolata, A. formosa, A. reticulosa and Litheusphaerella spectabilis. The X-ray analysis confirmed that specimens identified as archaeomonads possessed walls of silicon.

Additional records of archaeomonads in water samples were obtained from a search of our data files on epipelagic organisms found in the central California Current region and from a 1,650-m water sample (provided by A. Alldredge) from the same area. These records indicate that archaeomonads occurred in two locations from the central California Current at 35°70′ N, 123°67′ W, 50 m and 32° N, 118° W, 1,650 m (M.W.S. unpublished data). Although very rare in these samples, two different species were present, A. mangini and A. striata.

Four sea-ice samples were taken from the same area as the Weddell Sea water samples, one during the 1978 IWSOE and

Table 1 Coordinates for locations shown in Fig. 1

Deep Sea Drilling Project sediment samples						
Site	Lat.	Long.	Series	Depth		
Leg 12						
112	54°01′ N	46°36′ W	Upper Pleistocene	3,657		
Leg 18						
178	56°57′ N	147°08′ W	Upper Pleistocene	4,218		
182	57°53′ N	148°43′ W	Upper Pleistocene	1,411		
Leg 19						
183	52°34′ N	161°12′ W	Upper Pleistocene	4,708		
186	51°07′ N	174°00′ W	Upper Pleistocene	4,522		
188	53°45′ N	178°40′ E	Upper Pleistocene	2,649		
190	55°33′ N	171°38′ E	Upper Pleistocene	3,875*		
191	56°56′ N	168°10′ E	Upper Pleistocene	3,854		
193	45°48′ N	155°52′ E	Upper Pleistocene	4,811		
Leg 28						
266	56°24′ S	110°07′ E	Upper Pleistocene	4,167		
268	63°57′ S	105°09′ E	Upper Pleistocene	3,529		
269	61°41′ S	140°04′ E	Quaternary	4,282		
270	77°26′ S	178°30′ E	Pliocene-Recent	663*		
271	76°43′ S	1 75° 03′ W	Quaternary	562*		
272	77°08′ S	176°46′ W	Lower Pliocene	619*		
273	74°32′ S	174°38′ E	Quaternary-Pliocene	491*		
Leg 35						
323	63°41′ S	97°58′ W	Upper Pliocene	5,004		
325	65°03′ S	73°40′ W	Upper Pliocene	3,748		
Leg 36						
326	56°35′ S	65°18′ W	Upper Pleistocene	3,812		
327	50°52′ S	46°47′ W	Upper Pleistocene	2,400		
328	49°49′ S	36°40′ W	Upper Pleistocene	5,095		
Leg 38						
340	67°12′ N	06°18′ E	Upper Pleistocene	1,217		
344	76°09′ N	07°52′ E	Pleistocene	2,156		
348	68°30′ N	12°28′ W	Upper Pleistocene	1,763		

Scripps Institution of Oceanography repository sediment samples

Lat.	Long.	Depth (m)				
50°49' N	166°35′ W	5,025				
12°37′ S	78°39′ W	5,960				
14°18′ S	78°18′ W	4,300				
17°38′ S	16°12′ W	3,427				
48°13′ N	157°26′ W	5,220				
51°34′ N	149°58′ W	4,484				
0°55′ N	104°12′ W	3,496				
1°00′ N	113°42′ W	3,947				
1°01′ N	124°33′ W	4,771				
0°59′ N	135°05′ W	4,297				
64°03′ S	61°39′ W	1,050*				

^{*}Archaeomonads present.

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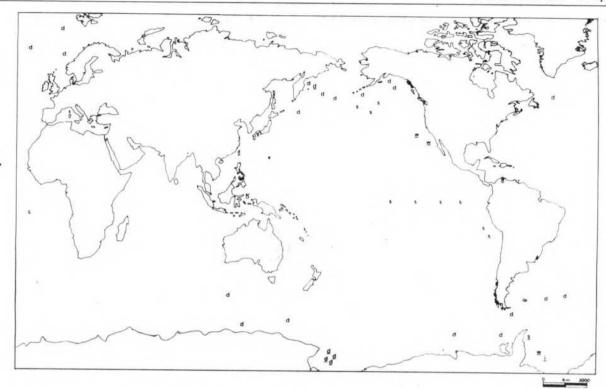


Fig. 1 The distribution of samples examined. d, Deep Sea Drilling Project samples; s, Scripps Institution of Oceanography samples; w, water column samples; i, ice samples. Underlined letters indicate the locations where archaeomonads were present.

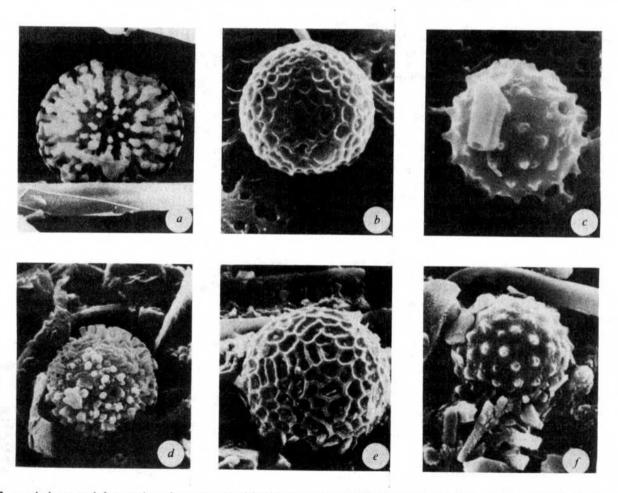


Fig. 2 a-c, Archaeomonads from sea-ice and water samples. d-f, Archaeomonads from sediment samples. Litheusphaerella spectabilis Deflandre (a and d). Archaeomonas areolata Deflandre (b and e). A. mangini Deflandre (c and f). Other archaeomonads tentatively identified to species were: A. striata Deflandre, A. sphaerica Deflandre, A. reticulosa Deflandre and A. formosa Deflandre. See the text for exact distributions. Perch-Nielsen^{16,17} and Deflandre⁶ provide species descriptions and scanning electron micrographs of the above species, and Stradner² the ultrastructural information on A. areolata. Magnifications ×6,500 and background pores in b and c are 0.4 µm in diameter.

three during the 1980 IWSOE. After the ice was washed and melted, the ice microorganisms were prepared in the same way as those from the water column. The 1978 sample was quantitatively prepared and counted, whereas those from the 1980 season were not; thus the 1980 material provides only qualitative data. Archaeomonads were present in all four samples of ice, and they occurred in densities of $13 \times 10^3 \, l^{-1}$ in the 1978 material and also were abundant, relative to diatom frustules, in the 1980 samples. Four species were found in Weddell Sea ice from the two years, A. areolata, A. formosa, A. mangini and

Thirty-six samples of surface marine sediment were examined (Table 1, Fig. 1) from areas throughout the world ocean where siliceous sediments are presently being deposited14. Twenty-five sediment samples were obtained from the Deep Sea Drilling Project repository, and 10 from the core library at Scripps Institution of Oceanography. The remaining sample was from a gravity core taken in the Weddell Sea (64°03' S 61°39' W). Thirty-one sediment samples range from Upper Pleistocene to Recent; three sediment samples are Pliocene, and two samples were dated as no older than the Pliocene (Table 1).

Cysts were obtained by suspending sediment aliquots in vials of distilled water, dispersing the slurry using a vortex mixer, and allowing the coarser fraction to settle for 5-7 min after which the upper third of the water was decanted, filtered and the filter treated for SEM. To avoid bias in the results, the samples were processed so that during the examination we did not know the origin of the sediment.

Archaeomonads were found in 6 out of the 36 sediment samples examined (Table 1). Five of the six samples were from south of the Antarctic Circle. One came from the Weddell Sea, four from the Ross Sea and one sample from the Western Bering Sea (55°33' N, 171°38' E). Six species of archaeomonad were found in the Ross Sea sediment samples, A. areolata, A. formosa, A. mangini, A. reticulosa, A. sphaerica and L. spectabilis. Two species were found in the Weddell Sea sediment sample A. areolata and A. striata. As the samples without archaeomonads were searched as carefully as those containing the cysts, we think the lack of these specimens in the other samples indicates a real decrease in their abundance outside the areas of sea ice.

The discovery of significant numbers of archaeomonads in sea ice, water underlying the ice, and surface sediments underlying areas often covered with ice (Fig. 2) represents the first finding of archaeomonad-like cysts in the modern ocean. Their appearance in these samples establishes continuity from early Pleistocene archaeomonads to Recent marine chrysophyte cysts, and the modern distribution implies a distinct association between modern archaeomonads and sea ice. Although these cysts occurred in low numbers elsewhere, as shown by their presence in the California Current water samples, they were insufficiently abundant to contribute to underlying sediments. Similar sea-ice associations have been reported by Sancetta¹⁵ who found particular abundance ratios of diatom species in sediments associated with overlying ice and hydrographic conditions. Although chrysophytes occur throughout the world's oceans, they may, like their freshwater relatives9, produce a significant number of cysts only in extreme environmental conditions.

The distribution and abundance data presented here suggest that, in response to sea-ice formation, significant numbers of chrysophytes form the resistant cysts described previously as archaeomonads. This response to sea-ice coverage, which is a limited stimulus on a global scale, may explain why archaeomonads are absent or rare in most siliceous sediments and suggests other locations where they might be found. The association of archaeomonad cysts with polar sea ice, indicates they may be useful palaeoclimatological indicators.

We thank D. Ringo for encouragement and suggestions throughout the project. L. LaPorte, C. Moore, P. Bolt, E. Chu, and E. Diaz de Leon provided helpful comments. J. Nowell, M. Gowing and C. Zenker provided valuable discussion and

help with the SEM, M. Gowing took micrograph Fig. 2c. We thank D. Adam for the X-ray analysis and for preprints. W. Reidel provided the Weddell Sea sediment sample. T. Wood and T. Walsh provided samples. We also thank T. Foster for access to samples, equipment and ship time. J.G.M. thanks A. and C. Pert. Funding was provided by a UC President's Undergraduate Fellowship, other internal awards and NSF grants DPP 75-14936, DPP 78-07797 and DPP 80-20616.

Received 2 November 1981, accepted 2 February 1982.

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Earliest floral evidence for the Ebenaceae in Australia

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Flowers are seldom preserved in an identifiable condition. The limited knowledge of floral structure of ancient flowering plants makes comparisons with living plants difficult, as floral structure is the basis for classification of the angiosperms. When found, fossil flowers contribute considerably to our understanding of the evolution of flowering plants¹⁻⁶. The flowers described here were recovered from Upper Eocene deposits near Anglesea, Australia. They are tetramerous, unisexual, male, with 16 stamens and tricolporate pollen. These flowers, and associated ebenaceous leaves, are the oldest known remains of the Ebenaceae from Australia.

The Ebenaceae, the persimmon and ebony family, represents one of the small group of families of angiosperms whose name is not based on a currently valid generic name. The familial name was derived from the pre-Linnaean Ebenus Rumphius. Generic limits within the family are the subject of some controversy. As many as nine genera, including the widely used Maba Forst., Royena L, and Tetraclis Hiern have been recognized, but a more recent consensus has included all but one of these, Euclea Murr., in Diospyros L, the currently valid type genus⁷⁻⁹. Of the 400-500 species ascribed to the Ebenaceae, only 14 may be attributed to Euclea, with the remainder in Diospyros sensu lato. Generic segregation was rejected due to the great uniformity of floral structure throughout Diospyros s.l.

The Ebenaceae is pan-tropical, with a few temperate outliers such as the American persimmon, Diospyros virginiana L. By far the greatest diversity is found in the Indo-Malaysian region. No recent synopsis of Australian species is available, but published regional floras indicate that 15 species of Diospyros occur

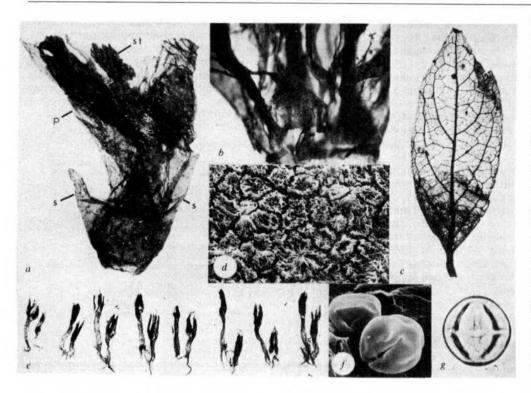


Fig. 1 Fossil flowers and leaves from the Eocene of Anglesea, Australia. a, Complete flower showing sepals (s), petals (p) and stamens (st), specimen number A-340 in the University of Adelaide Paleobotanical Collection (×7) b, Rudimentary gynoecium, surrounded by filaments of stamens, at centre of flower, A-342 (×25). c, Fossil leaf found in association with flowers, AM-243 (×1.4). d, Scanning electron micrograph of abaxial surface of leaf; note cuticular thickenings over epidermal cells and the overarching papillae that obscure the stomata, e, Stamens AM-260 $(\times 365).$ removed from a complete flower, arranged in their relative position within the flower; note that stamens occur in pairs with one short and one long filament, A-341 (×5). f, Scanning electron micrograph of a fossil pollen grain, in polar view, preserved within an anther; note the smooth appearance of the exine (×450). g, Light micrograph of a fossil pollen grain from an anther; equatorial view showing tricolporate structure (×450).

in Australia^{10,11}. Although many stellate calyces and entiremargined leaves of late Cretaceous, and Tertiary age have been assigned to *Diospyros*, in few instances are features well enough preserved or of sufficient diagnostic value for positive identification. The fossil record for leaf and especially floral structure of the Ebenaceae is scant.

The fossils described here were found in clays overlying the Eastern View Coal Measures exposed at the Alcoa power station at Anglesea, Victoria. The sequence includes coarse to fine cross-bedded sands with lenses of carbonaceous clay, and has been dated as late Eocene^{12,13}. Most of the ebenaceous fossils occur in a single clay lens in association with a diverse leaf flora of over 40 taxa. Previous studies of clay lenses about 8 m higher in the sequence have revealed the presence of Casuarinaceae, *Bowenia*, and a zamioid cycad, *Pterostoma* Hill¹⁴⁻¹⁶.

Approximately 100 flowers have been recovered. All that remains of most of them are four-parted calyces whose sepals are fused in their basal third. Total sepal length is 3-5 mm and width is ~ 2 mm, and their shape is roughly triangular. Sepals possess numerous simple hairs on both inner and outer surfaces. The corolla and sexual organs seem to have been lost from the calyx as a unit. This is supported by the presence of such units in the macerates. Complete flowers and isolated corolla units contain only stamens and were strictly unisexual, male flowers. A small, bulbous structure at the centre of a few flowers represents an aborted gynoecium (Fig. 1b).

The actinomorphic corolla consists of four contorted petals that alternate with the sepals. The petals are fused to just above the tips of the sepals, a distance of about 1/3 of their total length of 7–9 mm. Petals, like sepals, have unicellular, simple hairs, although hairs tend to be most abundant on the margins and abaxial midline.

The androecium consists of 16 stamens which are paired, each pair comprising one long- and one short-filamented anther (Fig. 1e). Each anther has four locules and dehisces by longitudinal slits. Bleaching of the fossil anthers often causes separation of the tapetum from the anther wall, freeing four pollenfilled pouches from each. The pollen is tricolporate, subprolate, and $\sim 30~\mu m$ in equatorial diameter (Fig. 1f, g). The grains have a smooth to slightly scabrate exine.

No female flowers have yet been recovered. However, the observed rudimentary gynoecia are sufficient to illustrate that the flower is hypogynous, with an ovary with more than one style. Unisexuality in flowering plants is commonly achieved by such incomplete development of one type of sex organ. The clustering of well-preserved fossil flowers may indicate that they are derived from one or a few individuals that grew very close to the site of deposition. Most living *Diospyros* are dioeceous, and a similar condition in the fossil species may explain the abundance of male and apparent lack of female flowers.

Floral and pollen morphology of the fossil flowers form a unique suite of characters found only in one extant family, the Ebenaceae. Favourable comparisons may be made with many extant species of *Diospyros* s.l. *Diospyros* is a closely knit genus with remarkably uniform floral structure. Although flowers are tri-, tetra-, to pentamerous, the general appearance of the unisexual flowers, with their calyx cup, connate and contorted petals, radial pairs of stamens, tricolporate pollen, and superior ovary, varies little. The presence of eight radial pairs of stamens in the fossil and in many tetramerous extant *Diospyros* is very unusual among flowering plants and in itself could be considered diagnostic. Only minor differences in shape and size of floral organs, and no significant structural differences, distinguish the fossil flowers from such living tetramerous species of *Diospyros* as *D. australis* Hiern.

Associated with the fossil flowers are leaves of more than 40 species of angiosperms. The commonest of these, currently represented by 45 complete individuals in our collection, is a

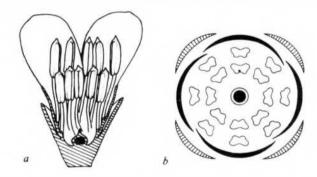


Fig. 2 a, Reconstruction of flower in median longitudinal section. b, Floral diagram.

highly variable taxon. It ranges in size from 4 to 110 mm in length and 2 to 60 mm in maximum width. Shape is equally variable, ranging from lanceolate to ovate to nearly orbicular. Venation is brochidodromous (Fig. 1c). These features are consistent with several extant Australasian species of Diospyros, but are by no means diagnostic of them. Of greater importance is the cuticular structure of these fossil leaves. The stomatal complexes are of the actinocytic type, with 4-6 irregular subsidiary cells. Each subsidiary cell produces a large papilla that overarches the guard cells. Cells of the lower epidermis possess extensive ridge-like thickenings of the cuticle (Fig. 1d).

Cuticular patterns on the abaxial epidermis of extant Diospyros are extremely diverse, ranging from nearly smooth (such as D. calycantha O. Schwarz), to striate or wrinkled, to ridged, to ornate, with large, frill-like accumulations of cutin (such as D. australis Hiern, D. macrocarpa Hiern). Due to intraspecific consistency of characteristic epidermal modifications, cuticular analysis is a valuable tool in identification and classification of both living and fossil plants. Cuticular thickenings, subsidiary cell arrangement, and over-arched stomata of the fossil leaves are similar to many extant Diospyros and fit easily within the range seen in the genus.

Of great significance is the occurrence of the same distinctive cuticular pattern on the fossil leaves and on the sepals of the fossil ebenaceous flowers. Flowers and leaves have not been found in organic connection. However, close association in the fossil flora, similarity of foliar and floral features to living Ebenaceae, and especially cuticular similarity of both organs indicate that the fossil leaves and flowers represent one taxon.

The evolutionary history of the Ebenaceae is unclear. There is a general consensus that the Ebenales was derived from the Theales, but the divergence has been significant 17,18. Many fossils (both leaves and fruits) have been ascribed to Diospyros. They are reported from North America, Europe, and Asia as well as Australia 19-22. Ettingshausen reported Diospyros leaf impressions and fruit casts from Australia²³. However, with no floral structure and no cuticles, his identifications are questionable. Similarly, serious doubt is cast on other reports of macrofossil evidence of the family. Ebenaceous pollen is not distinctive, and Palaeogene reports are unreliable. The flowers reported here are the earliest known identifiable floral remains of the Ebenaceae and the earliest well documented occurrence of the family in Australia.

As the Ebenaceae is pan-tropical, with the centre of diversity in Malaysia, it is tempting to believe that this might have been the region of origin. If this were true, the 15 extant Australian species would most probably have evolved from post-Miocene arrivals through New Guinea, since Palaeogene dispersal from South-east Asia to Australia would invoke excessive, if not incredible distances.

A Malaysian origin is opposed by the many species in Australia, South America, and Africa, and the Eocene fossils from Australia, which suggest that the Ebenaceae had a Gondwana origin with subsequent dispersal to the Malaysian region, possibly as late as the Miocene, through India and/or Australia. However, if some of the Laurasian fossils of early Tertiary age, in particular flowers assigned to Diospyros from the British late Eccene²², are correctly assigned to the Ebenaceae, the family may have already become very widespread by that time.

The Anglesea fossils also provide evidence that the floral features typical of many Ebenaceae—for example, tetramery, unisexuality, stamens in pairs and four times the number of corolla lobes, fused and contorted petals, and tricolporate pollen-were well established by Eocene times, and that selective pressures since that time have not been sufficient to alter them. Foliar features, too, were well advanced. Leaf architecture and the peculiar cuticular thickenings that characterize living Ebenaceae were present in the Eocene and were already associated with a typically ebenaceous floral plan.

Systems of angiosperm classification continue to be based, as they have since the time of Linnaeus, on floral morphology.

The validity of phylogenetic interpretations based on these systems is tested most critically by floral evidence from the fossil record. Within this framework, the importance of the discovery and analysis of structurally preserved flowers, such as those from Anglesea, is obvious.

We acknowledge the support of Alcoa of Australia, the Australian Research Grants Committee for grant E77/155 E 2R to D.C.C., and of the Natural Sciences and Engineering Research Council of Canada for a fellowship to J.F.B.

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Self-excitation of olfactory bulb neurones

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The concept that neurotransmitter released from a neurone may feed back and influence the excitability of the same neurone has been suggested by a variety of evidence. Anatomical studies have shown that axon collaterals can arborize among the dendrites of the parent neurone¹⁻⁷, suggesting a direct feedback via axon collaterals. In addition, the dendritic release of dopamine from substantia nigra neurones has suggested that dopamine may exert a direct feedback inhibition of these neurones^{8,9}. Little electrophysiological evidence is available, however, to indicate that such a mechanism does exist. Based on intracellular recordings, Park et al. 10 have proposed a direct inhibitory feedback in the neostriatum, but a presynaptic mechanism was not entirely excluded. We have now found that blockade of synaptic inhibition of relay neurones in the olfactory bulb unmasks long-lasting depolarizing potentials which can trigger repetitive discharges. These depolarizing potentials result from direct feedback of dendritically released excitatory transmitter onto the same and neighbouring relay neurones. Such a process might contribute to epileptogenic neuronal discharge.

We have used the *in vitro* turtle (*Pseudemis scripta elegans*) olfactory bulb preparation¹¹⁻¹³. The bulb was hemisected to facilitate the placement of stimulating and recording electrodes. Microelectrodes (140-180 M Ω) filled with 2 M potassium methylsulphate were used to record intracellularly from mitral cells (the principal relay neurones of the olfactory bulb). Mitral cells could be activated synaptically by stimulating the olfactory

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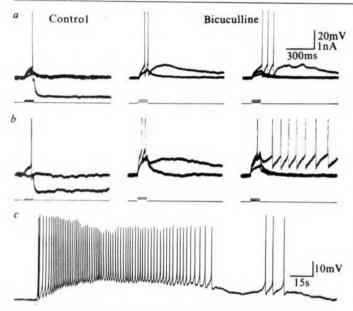


Fig. 1 Blockade of i.p.s.ps reveals depolarizing after-potentials. The superimposed oscilloscope records in this and the following figures show one or two traces with subthreshold depolarizing current pulses applied through the recording microelectrode and one trace with a suprathreshold pulse. The current trace is shown below the voltage trace. a, b Show two examples of the effect of bicuculline methiodide (BMI) (0.1 mM). The GABA antagonist had been present for 8 min during the second and third responses in a, and for 10 min in b. The cells often fired repetitively during the depolarizing after-potential. Resting membrane potential for cell in a is -57 mV and in b, -68 mV. Spikes in b are truncated. c Shows a pen record of a prolonged burst of action potentials elicited by a single 100-ms depolarizing current pulse. This cell had been exposed to BMI (0.2 mM) for 17 min. Resting membrane potential -52 mV. The calibration in a also applies to b.

nerves, antidromically by stimulating their axons or directly by passing depolarizing current pulses through the recording electrode. A bridge circuit was used so that the membrane potential could be monitored during the current pulses.

In the absence of an antagonist of γ -aminobutyric acid (GABA), action potentials evoked in mitral cells by the injection of depolarizing current were followed by inhibitory postsynaptic potentials (i.p.s.ps) (Fig. 1a, b) which result from the direct activation of the reciprocal dendrodendritic synapses between mitral and granule cell. Similar to the i.p.s.ps elicited by stimulating mitral cell axons 12-14, these directly activated i.p.s.ps are thought to be mediated by GABA12,13. Addition of the GABA antagonists, bicuculline methiodide or picrotoxin, to the superfusate blocked the hyperpolarizing i.p.s.p. and, in 40 of 46 cells examined, uncovered a prolonged depolarizing after-potential (DAP) (Fig. 1a, b) which was at times intense enough to trigger repetitive firing of the mitral cell (Fig. 1). In Fig. 1c, a single 100-ms depolarizing current pulse evoked a discharge lasting for ~3 min. We considered three possible mechanisms for this DAP. It could result from an increase in a sodium conductance which inactivates slowly, such as has been reported in cerebellar Purkinje cells¹⁵. However, unlike the Purkinje cell response, the mitral cell DAP was not blocked by the addition of a concentration of tetrodotoxin (TTX) (1 µM) sufficient to block the fast directly evoked action potential (n = 11) (Fig. 2).

Another process which could produce the DAP is a slow, voltage-dependent calcium conductance. The DAP is calcium dependent because in the seven cells examined it was blocked by the calcium antagonists cobalt (2 mM) or cadmium (0.5 mM), which also blocked the TTX-insensitive action potential (Fig. 2; see also refs 11-13). The calcium antagonists would also be expected to block the release of transmitter. If the DAP were a result of a voltage-dependent mechanism (whether calcium or sodium mediated) it should be aborted by hyperpolarizing

the membrane, as has been seen in other systems $^{16-18}$. However, injection of a hyperpolarizing current pulse through the recording microelectrode did not alter the duration of the DAP (n=6) (Fig. 3a) even when the hyperpolarizing pulse outlasted the DAP. This finding suggests that the DAP may result from feedback of excitatory transmitter released from the same mitral cell.

Another manipulation that might distinguish between a calcium conductance increase and a feedback of excitatory transmitter is the substitution of barium for calcium. Barium can readily pass through calcium channels (in some cases better than calcium19), but it substitutes poorly, if at all, for phasic evoked transmitter release²⁰⁻²³. We have tested the ability of barium to substitute for calcium in synaptic transmission in the olfactory bulb by recording the field potential evoked by the excitatory synaptic action of mitral cell dendrites on granule cell dendrites. We found that when synaptic transmission was blocked in a zero calcium Ringer, 3 mM barium was unable to restore transmission. However, switching back to normal Ringer containing 3 mM calcium quickly restored the synaptic potential. The effect of barium substitution on the DAP was examined in five cells. A typical result is shown in Fig. 3b in which the DAP was completely blocked while the amplitude of the TTX-insensitive spike was actually increased. The abolition of the DAP by barium substitution suggests that the DAP is mediated by the release of synaptic transmitter. Note that addition of barium to normal Ringer did not block the DAP13, indicating that barium is not a calcium antagonist for the release process, but rather fails to mimic the action of calcium. In light of the evidence presented above and because the DAP was not blocked by deleting axonal pathways with TTX, we suggest that mitral cell activation causes dendritic release of an excitatory transmitter which feeds back directly onto the same mitral cell.

Recent biochemical evidence strongly suggests that aspartate is the transmitter released from mitral cell axon terminals in the olfactory cortex²⁴. It is, therefore, reasonable to expect that aspartate is the transmitter released from mitral cell dendrites. We have found that iontophoresis of either glutamate or aspartate in the presence of GABA antagonists depolarizes mitral cells²⁵. α -Amino adipate (α AA), which antagonizes aspartate responses in other systems^{26,27}, decreased the depolarizing

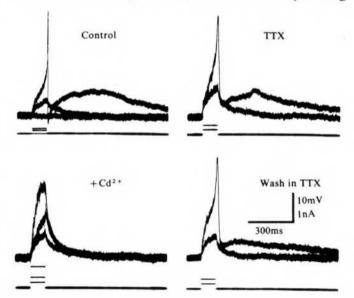


Fig. 2 The depolarizing after-potential is resistant to tetrodotoxin (TTX) but blocked by the calcium antagonist cadmium. The control record was obtained 10 min after switching to bicuculline (0.1 mM) (spike truncated). TTX (1 μM) was then superfused for 22 min and only slightly reduced the DAP. After exposure to cadmium (Cd²⁺) (0.5 mM) for 10 min, the depolarizing after-potential and the calcium spike were entirely blocked. Washing for 90 min (in TTX) restored the calcium spike and DAP. Resting membrane potential, -56 mV.

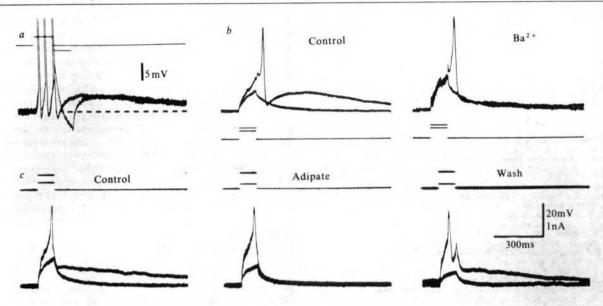


Fig. 3 Properties of depolarizing after-potential. In a, a hyperpolarizing current pulse applied immediately after a suprathreshold depolarizing current pulse did not diminish the DAP. The preparation had been bathed in BMI (0.1 mM) for 23 min. Resting membrane potential, -53 mV. The control record in b was obtained 30 min after switching to a solution containing TTX (1 μM) and BMI (0.1 mM). The record to the right was obtained 12 min after substituting 3 mM barium for calcium. Resting membrane potential, -57 mV. The control record in c was obtained 50 min after switching to a solution containing TTX (1 μM) and picrotoxin (0.2 mM). Adipate (1 mM) was then added to the superfusion medium for 15 min. The record on the right was obtained 24 min after returning to the control solution. Resting membrane potential, -62 mV. The calibration in c also applies to b.

effect of iontophoretic aspartate on mitral cells (not shown). We examined the sensitivity of the DAP to α AA in the presence of TTX so that we could use the calcium spike as a monitor of the specificity of α AA. The DAP was reversibly abolished by α AA (n=6), which had little or no effect on the calcium spike (Fig. 3c). The blocking effect of α AA, therefore, occurred after calcium entry. The i.p.s.p. in normal Ringer was also reversibly abolished by α AA.

After inhibition was blocked, antidromic action potentials were also followed by DAPs (Fig. 4b). The antidromic spike can be fractionated by passing a hyperpolarizing current pulse through the recording microelectrode, timed to occur when the antidromic spike arrives at the soma 12,13. Large current pulses blocked invasion of the soma-dendritic membrane, revealing a small action potential that probably resides in the initial segment^{28,29}. When only the initial segment was activated, the resulting DAP was small (Fig. 4b). However, when invasion of the soma-dendritic membrane occurred, a larger DAP resulted. This incremental increase in the amplitude and duration of the DAP is, therefore, due to activation of the soma-dendritic membrane. A depolarization could also be evoked by antidromic stimulation which was subthreshold for the axon of the impaled cell (Fig. 4a). These findings suggest that transmitter released from antidromically invaded mitral cells can diffuse to neighbouring mitral cells. Indeed, in the presence of GABA antagonists, mitral cells frequently fire spontaneous bursts of action potentials (Fig. 4c), suggesting the presence of synchronized activity.

Thus, in normal conditions an excitatory transmitter, probably aspartate, is released from mitral cell dendrites and activates receptors on the processes of granule cells. The ensuing depolarization results in the release of GABA from the granule cells back onto the same mitral cell. When the inhibitory half of the reciprocal synapse is blocked with GABA antagonists, the activation of olfactory bulb mitral cells is followed by an excitatory depolarizing potential instead of an i.p.s.p. The present findings indicate that this depolarizing potential results from the direct feedback of dendritically released excitatory transmitter onto the same mitral cells. The depolarization elicited by both subthreshold antidromic stimulation and in the presence of only an initial segment spike indicates that this phenomenon is not limited to single cells, but can involve an interaction of

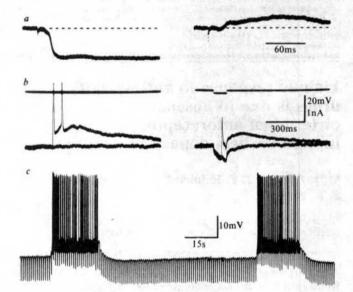


Fig. 4 Antidromic stimulation evokes depolarizing responses in the presence of GABA antagonists. The response on the left in a shows an i.p.s.p. to a stimulus below threshold for the axon of the impaled cell. The right-hand record was obtained 32 min after switching to a solution containing 0.2 mM picrotoxin. Resting membrane potential, -64 mV. The responses in b were obtained 12 min after switching to a solution containing 0.1 mM bicuculline methiodide. The record on the left in b shows the response to antidromic stimulation suprathreshold for the impaled cell (spikes truncated). The record on the right shows that when a hyperpolarizing current pulse blocked firing of the soma-dendritic membrane, the DAP was reduced in size but was still present. Resting membrane potential, -58 mV. c Shows spontaneous bursts of action potentials from the same cell illustrated in a, 47 min after switching to the picrotoxin-containing solution. The voltage gain in a is twice that in b.

a population of neurones, each contributing to the depolarization of its neighbours. In addition, excitatory recurrent collaterals could also contribute to this lateral spread of excitation³⁰. The prolonged depolarization which follows olfactory nerve stimulation in the presence of GABA antagonists^{13,31}

may also be due in part to similar excitatory feedback mechanisms. While the physiological significance of self-excitation, either via a dendritic or axon collateral pathway, is unclear, such a process might well contribute to epileptogenic burst discharge in other brain regions.

This research was supported by NIH grant NS 16485, RCDA NA 00287 to R.A.N. and the Klingenstein Fund.

Received 17 November 1981, accepted 12 February 1982

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Unique response to antipsychotic drugs is due to absence of terminal autoreceptors in mesocortical dopamine neurones

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A projection of dopamine (DA) neurones has been identified which runs from the rat midbrain ventral tegmental area to the cerebral cortex1. Relative to other cortical areas, the prefrontal cortex is enriched in DA^{2,3}, tyrosine hydroxylase³, DA uptake^{2,3}, DA-sensitive adenylate cyclase² and DA-responsive neurones4. Lesions of the prefrontal cortex or selective lesions of the prefrontal cortical DA innervation induce functional deficits, including detrimental effects on delayed response tasks, a difficulty in suppressing attention to irrelevant stimuli and a diminution in affective and social behaviour (for a review, see ref. 5). Recent studies have shown that this mesocortical DA system differs from other DA systems (such as the nigrostriatal and mesolimbic DA systems) in terms of drug responsiveness. For example, the acute administration of antipsychotic drugs (for example, haloperidol) greatly accelerates DA turnover in the nigrostriatal and mesolimbic DA systems through multiple sites of action, but only modestly accelerates mesocortical DA turnover. Following the chronic administration of moderate doses of antipsychotic drugs, tolerance to the effects of a haloperidol challenge dose is generally seen in the striatum and olfactory tubercle but not in the frontal cortex⁶⁻⁸. Similar results obtained in experiments involving primates have led to the suggestion that the frontal cortex may be the site of therapeutic

action of the antipsychotic agents^{9,10}. In light of the recent finding that rat mesocortical DA neurones lack terminal autoreceptors¹¹, we have investigated the possible role of nerve terminal DA autoreceptors in mediating the varied responses seen in different brain regions following acute and chronic antipsychotic drug treatment. The results, reported here, suggest that both the relatively small activation of the mesocortical DA system in response to a haloperidol challenge, and the lack of tolerance to haloperidol following chronic treatment, may be related to the absence of nerve terminal autoreceptors.

Male Sprague-Dawley rats (Charles River; 200-250 g) were injected intraperitoneally (i.p.) with either haloperidol (0.5 mg per kg) or vehicle for 28 days, as previously described¹². Some animals received a haloperidol challenge (1 mg per kg) or vehicle 60 min before decapitation. For experiments directly assessing DA autoreceptor function, other rats were withdrawn from haloperidol for 7 days before use. In other experiments, unilateral knife cuts of the medial forebrain bundle were performed under halothane anaesthesia 35 min before decapitation. The coordinates used were: 3,180 µm anterior to lambda; 500 µm lateral to the midline; 6,000 µm vertical from the dura¹³. From this position the knife was extended ~4 cm and lowered to the base of the brain. The striatum, olfactory tubercle and prefrontal cortex were dissected from the brain¹¹, and DA and dihydroxyphenylacetic acid (DOPAC) levels determined chromatography using liquid with electrochemical detection11,14

Acute haloperidol challenge dramatically increased both striatal and olfactory tubercle DOPAC levels (Fig. 1). In the prefrontal cortex, a much smaller but significant increase in DOPAC was seen after haloperidol challenge (Fig. 1). In parallel experiments, haloperidol challenge greatly accelerated the α -methyltyrosine-induced decline of DA in the striatum and olfactory tubercle, but not that in the prefrontal cortex (M.J.B. and R.H.R., unpublished). It has been previously demonstrated that the small haloperidol-induced increase in prefrontal cortical DA synthesis is totally prevented following the blockade of dopaminergic impulse flow¹¹. As mesocortical DA neurones lack nerve terminal autoreceptors, it has been

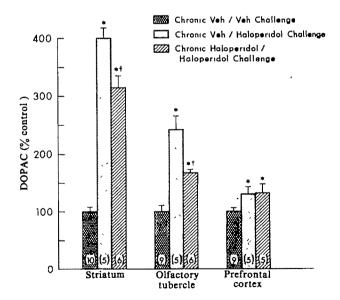


Fig. 1 Effects of acute and chronic haloperidol treatment on DOPAC levels in various brain regions. Animals were chronically treated with haloperidol or vehicle as described in the text. Haloperidol (1 mg per kg, i.p.) or vehicle challenge was administered 60 min before decapitation. The bars on the columns represent the s.e.m. and the numbers in the columns indicate n. Control striatal, olfactory tubercle and prefrontal cortical DOPAC levels were $1,092\pm86,1,004\pm96$ and 34 ± 2 ng per g respectively. *P<0.05 when compared with vehicle-treated animals, P<0.01 when compared with acute haloperidol challenge.

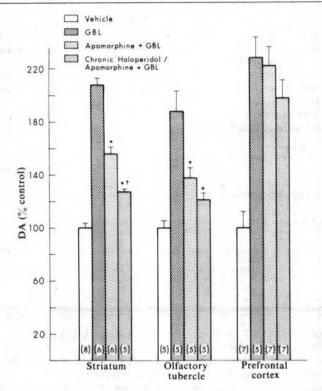


Fig. 2 Effects of chronic haloperidol treatment on DA autoreceptor sensitivity in various brain regions. Animals were chronically treated with haloperidol and withdrawn as described in the text. GBL (750 mg per kg, i.p.) was administered 35 min before decapitation. Some rats received apomorphine (0.25 mg per kg, i.p.) 5 min before GBL. The bars on the columns represent the s.e.m. and the numbers in the columns indicate n. Control DA levels in the striatum, olfactory tubercle and prefrontal cortex were $9,019\pm312$, $6,355\pm345$ and 86 ± 10 ng per g respectively. Chronic haloperidol treatment did not significantly alter DA levels (unpublished). *P<0.01 when compared with animals treated with GBL alone; $^{\dagger}P$ <0.05 when compared with the apomorphine-induced reversal of GBL in vehicle-treated rats.

suggested that the effects of haloperidol on this system are due solely to alterations in neuronal impulse flow¹¹.

Following chronic haloperidol treatment, significant tolerance to the DOPAC-elevating effect of a haloperidol challenge developed in both the striatum and the olfactory tubercle (Fig. 1). No change in the response to haloperidol was observed in the prefrontal cortex (Fig. 1). These results are in agreement with previous studies (in both rodents and primates) which have demonstrated the development of tolerance in the striatum, and in some cases limbic brain areas, with a lack of tolerance or even reverse tolerance in the frontal cortex⁶⁻¹⁰.

To examine the relationship between tolerance to haloperidol changes in autoreceptor sensitivity, a pharmacological method for eliminating dopaminergic impulse flow was used. Administration of γ-butyrolactone (GBL) reversibly blocks DA impulse flow, resulting in an increase in tyrosine hydroxylation and DA levels in nigrostriatal and mesolimbic DA systems similar to that seen after mechanical lesioning of these systems (for a review, see ref. 15). These changes have been proposed to result from a decreased DA autoreceptor activation secondary to the decreased amount of DA released into the synaptic cleft16. Direct-acting DA agonists prevent this increase in DA synthesis solely through the stimulation of DA nerve terminal autoreceptors^{11,12}. As previously reported, the GBL-induced increase in striatal and olfactory tubercle DA was partially reversed by an intermediate dose of apomorphine (Fig. 2). The increase in prefrontal cortical DA was unaffected by apomorphine pretreatment (Fig. 2), indicating the lack of mesocortical nerve terminal autoreceptors, as previously described11. Following chronic haloperidol treatment, an enhanced response to apomorphine, that is, DA autoreceptor supersensitivity, was directly demonstrated in the striatum (Fig. 2), confirming previous results ^{12,17,18}. The DA autoreceptors in the olfactory tubercle became slightly (but nonsignificantly) more sensitive, but no autoreceptor activity was revealed in the prefrontal cortex (Fig. 2).

To establish that the apparent lack of DA autoreceptors in the prefrontal cortex following GBL administration was not due to some unexpected effect of this drug on mesocortical neurones, autoreceptor function was assessed following the acute axotomy of the medial forebrain bundle (Fig. 3). Axotomy, like GBL, induced apomorphine-reversible increases in striatal and olfactory tubercle DA (Fig. 3), as previously reported 16,19,20. As expected, the axotomy-induced increase in prefrontal cortical DA was unaffected by apomorphine, providing additional evidence that mesocortical DA neurones lack terminal autoreceptors. The axotomy- and GBL-induced accumulation of prefrontal cortical DA (Figs 2, 3) are not a result of an acceleration of DA synthesis, but are presumably due to continued, normally rapid mesocortical DA synthesis for a period of time after the sudden blockade of impulse flow-coupled transmitter release 11,21.

DA neurones which lack nerve terminal autoreceptors show little (for example, the mesocortical system¹¹) or no (the tuberoinfundibular system²²) response to acute haloperidol challenge, while DA systems with terminal autoreceptors (for example, nigrostriatal, mesolimbic and tuberohypophyseal systems^{11,15,22}) respond dramatically. In the mesocortical system no tolerance to the effect of haloperidol challenge develops following chronic haloperidol administration, while tolerance does develop in the DA systems possessing nerve terminal autoreceptors⁶⁻¹⁰. Further, the time course for the development

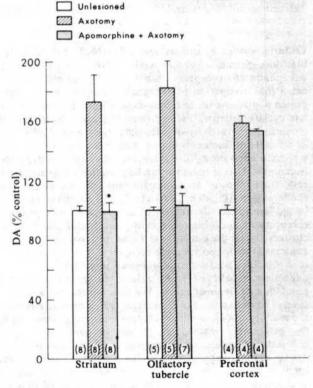


Fig. 3 Effects of apomorphine on nerve terminal DA autoreceptors after acute medial forebrain bundle axotomy. Lesions were made 35 min before decapitation using a retractable wire knife as described in the text. Some rats were pretreated with apomorphine (2 mg per kg, i.p.) 5 min before axotomy. The bars on the columns represent the s.e.m. and the numbers in the columns indicate n. DA levels in the unlesioned striata, olfactory tubercles and prefrontal cortices were $10,046 \pm 282, 4,948 \pm 115$ and 87 ± 3 ng per g respectively. * P < 0.001 when compared with animals receiving axotomies alone.

of tolerance in these systems may parallel the development of autoreceptor supersensitivity. These results suggest that the acute and chronic effects of haloperidol and other antipsychotic drugs on the various DA systems may be related to the presence or absence of nerve terminal autoreceptors. In addition, it has recently been determined that, in contrast to nigrostriatal and mesolimbic DA cells, mesocortical DA neurones innervating the prefrontal cortex also lack cell body DA autoreceptors (B. S. Bunney and L. A. Chiodo, personal communication).

Received 24 August 1981, accepted 4 February 1982

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The realization that mesocortical DA neurones lack both nerve terminal and cell body autoreceptors may aid in the development of potential therapeutic agents designed to interact selectively with discrete brain DA systems.

We thank Dr B. S. Bunney for his thoughtful comments throughout these experiments, Anne Morrison for laboratory assistance and Lynn Williams for secretarial assistance. This work was supported in part by USPHS grant MH-14092 and the state of Connecticut.

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Opioid peptides induce reduction of enkephalin receptors in cultured neuroblastoma cells

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Cultured mouse neuroblastoma cells (N4TG1)1,2 and neurocells (NG108-15)3-6 blastoma-glioma hybrid contain enkephalin (δ) receptors². Studies on hybrid cells have indicated that occupation of enkephalin receptors by opiates and opioid peptides leads to a time-dependent inhibition of adenylate cyclase activity3-5. If exposure of these cells to agonists is continued, the activity of adenylate cyclase gradually returns to normal and increases above normal on removal of opioids, a phenomenon thought to resemble tolerance and dependence in animals. Direct receptor binding studies on neuroblastoma cells have shown no internalization of receptors1. Using [D-Ala²,Lys⁶]Leu-enkephalin rhodamine-conjugated image-intensified fluorescence microscopy, enkephalin (δ) receptors in neuroblastoma cells were found slowly to form clusters which do not appear to be internalized. This is in contrast to many polypeptide receptors⁷⁻¹² which are internalized after the initial receptor-ligand interactions. This internalization is believed to be related to the so-called downregulation or desensitization. We now report that after one to several hours exposure of monolayer cell cultures to enkephalin, the number of enkephalin receptors measured subsequently in the extensively washed membrane preparations is greatly reduced. This reduction in receptor number seems to be selective for opioid peptides and is antagonized by a variety of opiate agonists and antagonists. These observations may be important in elucidating the phenomenon of opioid-induced desensitization.

Confluent monolayers of N4TG1 cells were pretreated with ligand, the cells were then washed extensively with Dulbecco's phosphate-buffered saline (PBS) to remove any remaining bound ligand and membrane particulate fractions were prepared. Enkephalin receptor binding of these fractions was then determined by measuring binding of ¹²⁵I-labelled [D-Ala²,D-Leu⁵]enkephalin (DADLE). Pretreatment of the cell monolayer for 4 h with 0.1 μM DADLE led to a large reduction in binding

of 125 I-DADLE to the subsequently prepared and washed membrane particulates. A similar loss of binding was observed for washed cells after pretreatment with DADLE; this reduction was time, temperature and dose dependent. At 37 °C, a significant reduction was observed after 30 min pretreatment, and had almost reached a maximum after 1 h. Further incubation only slightly increased the reduction (Fig. 1). Routinely we preincubated for 4h to ensure the maximum effect. At 24 °C, the reduction in binding was greatly decreased with respect to both the rate and extent of reduction (Fig. 1). The DADLE-induced reduction of binding is dose dependent (Fig.

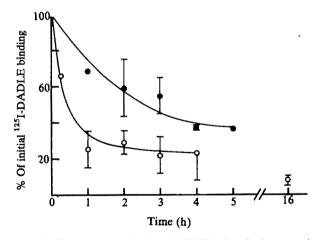


Fig. 1 Time course of the DADLE-induced decrease in ¹²⁵I-DADLE binding. N4TG1 cells were grown to confluence in Dulbecco's minimal essential medium supplemented with 75 U ml⁻¹ penicillin, 75 µg ml⁻¹ streptomycin sulphate and either 7% or 5% newborn calf serum (Gibco) in a 95% air/5% CO₂ humidified atmosphere at 37° C. DADLE was added to a final concentration of 10⁻⁷ M and the monolayers were incubated at 37 °C(O) or 24 °C (●) for the indicated times. After completion of the incubation, the medium was withdrawn, the monolayers were rinsed with PBS and the cells detached using 0.05% EDTA in PBS. The cells were pelleted by centrifugation, resuspended in PBS and incubated at 37 °C for 30 min to remove bound ligand. This incubation was repeated, then the cell pellet was resuspended in ice-cold 50 mM Tris pH 7.7 and the cells were homogenized using a Model PT-20 Polytron (setting 3.5, 20 s). The homogenates were centrifuged at 40,000g for 30 min and the resulting pellets were resuspended in 50 mM Tris pH 7.7 containing 100 mM NaCl; the suspensions were incubated at room temperature for 1 h. The membranes were again pelleted, resuspended and pelleted once in Tris buffer lacking sodium, and assayed for DADLE binding as previously described^{1,2}.

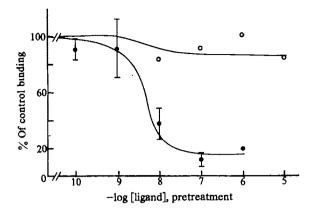


Fig. 2 Concentration dependence of the DADLE-induced decrease of enkephalin binding activity. Confluent monolayer cultures of N4TG1 cells were incubated with the indicated concentrations of DADLE (●) or morphine sulphate (○) for 4 h at 37 °C. Membrane fractions were prepared and binding activity was assayed as described in Fig. 1 legend. The data shown are the average values ±s.e.m. for two experiments.

2), having an EC₅₀ (concentration which causes 50% of the maximum reduction) of ~ 2 nM. This value is in reasonable agreement with the previously determined dissociation constant (K_d) of DADLE for the enkephalin receptor of this cell line^{1.2}.

The reduction in ¹²⁵I-DADLE binding activity induced by DADLE is specific for opiate receptors as this effect can be antagonized by the presence of an excess of opiate antagonists such as naloxone, nalorphine and diprenorphine (Table 1). These antagonists do not induce by themselves a reduction in ¹²⁵I-DADLE binding activity (Table 1). The natural endogenous peptides, Met- and Leu-enkephalin, as well as β -endorphin, [D-Ala²,NMePhe⁴,Met(O)ol⁵]enkephalin and [D-Ala²,Met⁵]enkephalinamide, also induce a reduction in ¹²⁵I-DADLE binding. Morphine and benzomorphan drugs such as ketocyclazocine and n-allylnormetazocine at high concentrations not only induce just a minimal reduction (usually ~20%) in the 125 I-DADLE binding activity (Table 1 and Fig. 2) but also antagonize the effect of opioid peptides (Table 1). This antagonistic effect of opiates against peptides is also dose dependent. Opiates are more effective against 0.01 µM DADLE than against a dose of 0.1 µM (Table 1), as would be predicted for competitive inhibition.

Incubation of cell monolayers with 10^{-8} M 3 H-DADLE at 37 °C for 4 h, followed by washing and preparation of membrane particulates in the same way as that used for the non-radioactive ligand, showed that 10% of the ligand initially associated with the cells remained bound. This corresponds to a concentration of DADLE $<10^{-10}$ M in the final assay. Because incubation of cells with 10^{-8} M DADLE caused a reduction of \sim 70% in the 125 I-DADLE binding (Fig. 2), the loss of binding activity could not be attributed simply to the competition of 125 I-DADLE with ligand that remained after preparation of the membrane fragments. Furthermore, diprenorphine, a ligand known to have an affinity greater than that of DADLE 13 , did not cause receptor reduction after similar pretreatment, suggesting that the peptide-induced receptor loss could not be explained simply by the high affinity of any ligand not washed away.

The enkephalin-induced reduction in binding is manifested by a decrease in the number of high-affinity binding sites, presumably enkephalin (δ) receptors. The saturation binding curves of ¹²⁵I-DADLE for control and 0.1 μ M DADLE-pretreated cell membranes showed a loss of total high-affinity binding sites (Fig. 3). The binding sites remaining after DADLE pretreatment had affinity similar to that of the control membranes. This was further confirmed by ¹²⁵I-DADLE competition binding studies using DADLE and morphine, in which we observed no significant difference in IC₅₀ values (concentrations

causing 50% inhibition of labelled binding) between control and pretreated membranes. As estimated from plots of log per cent ¹²⁵I-DADLE bound versus log concentration, the IC₅₀s for control and pretreated membranes respectively were 1.5 and 1.0 nm when DADLE was the displacing ligand and 35 and 75 nM for morphine sulphate, in good agreement with previous reports^{1,2,14}. However, it is unknown whether this reduction in the number of high-affinity binding sites is due to a loss of receptor sites or whether they are converted to sites of low affinity not detected in our binding assay. Recently, Bowen et al. ¹⁵ have reported an interconversion of μ and δ receptors in rat striatal patches that is mediated by divalent cations and sulphydryl reagents. Because pretreatment of N4TG1 cells with DADLE results in a loss of ³H-diprenorphine binding (a compound having similar affinity for μ and δ receptors) as well as ¹²⁵I-DADLE binding (data not shown), it seems unlikely that conversion from a δ to a μ form of receptor resulted in the observed down-regulation effect.

Numerous experiments in vivo and in vitro have indicated that enkephalin can mimic the biological effects of morphine, including the development of tolerance and physical dependence¹⁶. Previous experiments in vivo have led to the conclusion that neither the number nor the affinity of opiate receptors are altered after chronic treatment with morphine 17-20. We have confirmed this from measurements of the binding activities of three subtypes of opiate receptors including morphine (μ) , enkephalin (δ) and benzomorphan binding sites²¹ after chronic morphine treatment. Thus, the effect of reducing receptor numbers may be selective for opioid peptides and may differ from the phenomena of in vivo morphine tolerance and dependence. However, the recent demonstration of the lack of cross-tolerance between a δ -agonist (DADLE) and a μ -agonist (sulphentanyl) in mouse vas deferens after chronic in vivo treatment with either ligand²² suggests that there may exist tolerance selective for a receptor subtype. N4TG1 cells contain δ -receptors only. The present studies demonstrate that only opioid peptides induce a decrease in enkephalin receptors; morphine (μ -agonist), ketocyclazocine (κ -agonist) and N-allylnormetazocine (σ -agonist) have no such effect, in fact they antagonize the effects of opioid peptides. These observations resemble the studies on mouse vas deferens²². Selective

Table 1 Specificity of the ligand-induced decrease in enkephalin receptors

	Concen- tration	125I-DADLE binding (% of control ±s e m.) (n)		
Ligand	(M)	Ligand alone	+	
Agonists				
DADLE	10-7	19.0 ± 3.2 (8)		
	10-4	$27.6 \pm 3.5 (8)$		
Met-Enkephalin	10-6	$18.4 \pm 2.6(3)$		
Leu-Enkephalm	10 ⁻⁶	$17.3 \pm 2.2 (3)$		
D-Ala2,NMePhe4,		· · · · · · · · · · · · · · · · · · ·		
Met(O)ol ⁵ lenkephalin	10 ⁻⁶	$23.9 \pm 4.6(3)$		
[D-Ala2 Met5]-		• • • • •		
enkephalmamide	10-7	20.1 ± 0.7 (3)		
B-Endorphin	10^{-7}	$42.1\pm8.6(5)$		
Ketocyclazocine	10 ⁻⁵	$99.0 \pm 7.9(3)$	$55.8 \pm 2.8 * (2)$	
•			$72.7 \pm 15.0 \dagger (3)$	
Morphine sulphate	10-5	$83.7 \pm 8.2(3)$	$27.9 \pm 11.0 * (3)$	
	5×10^{-5}	$72.0 \pm 4.5 (4)$	$40.9 \pm 12.1*(3)$	
		, ,	$82.8 \pm 4.7 \uparrow (3)$	
N-Allylnormetazocine	10-5	$71.0 \pm 13\ 0\ (2)$	$89.3 \pm 9.7 + (3)$	
Antagonists				
Naloxone	10-5	91.3 ± 34.7 (2)	$96.7 \pm 19.1*(3)$	
Diprenorphine	10-7	77.3 ± 16.1 (3)	$72.5 \pm 13.8 \pm (3)$	
Nalorphine	10-5	81.8 ± 19.3 (3)	$96.5\pm2.6^{*}(2)$	

All ligands were preincubated with cells for 4 h before collection of the cells and preparation of membranes. As determined by Student's *t*-test, all values for peptides and morphine at 5×10^{-5} M differ significantly from the control (100%) (P < 0.005); all values for oplate agonists and antagonists except morphine at 5×10^{-5} M do not differ significantly from the control (P > 0.1). The values for oplates plus DADLE, except morphine (10^{-5} M) plus DADLE (10^{-7} M), differ aignificantly from the values for DADLE alone (P < 0.005)

*[DADLE] = 10^{-7} M

^{†[}DADLE] = 10⁻⁸ M

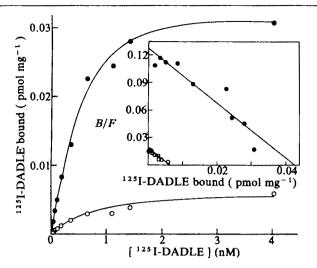


Fig. 3 Binding of 125 I-DADLE to N4TG1 membranes before and after preincubation of cells with unlabelled DADLE. Confluent cultures of N4TG1 cells were incubated with (O) or without (1) 10⁻⁸ M unlabelled DADLE and membranes were prepared as described in Fig. 1 legend Aliquots of the membranes were incubated with various concentrations of ¹²⁵I-DADLE for 1 h, after which time bound ligand was determined by rapid filtration through glass fibre filters. Nonspecific binding was determined in the presence of 10⁻⁵ M unlabelled DADLE. Inset, Scatchard plot of the data. The control membranes had a K_d of 0.76 nM and B_{max} of 42.8 fmol mg⁻¹, while the respective values for the treated membranes were 0.83 nM and 6.4 fmol mg⁻¹.

desensitization due to the loss of opiate receptors could occur for δ -receptors after chronic opioid peptide treatment.

There are many possible mechanisms for the ligand-induced loss of receptors reported here. For example, desensitized lowaffinity receptors may be induced by peptide which can no longer be detected by the present binding method. It is also possible that the receptors are cryptic after treatment with opioid peptides. The formation of (essentially) irreversible receptor-ligand complexes may also occur during the pretreatment. An understanding of the mechanism of receptor reduction may prove important in elucidating the physiological and pathological actions of opioids.

We thank Dr Pedro Cuatrecasas for reviewing the manuscript and for helpful discussion, and Mr Mark Collins for technical assistance.

Received 12 October 1981, accepted 3 February 1982

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Related amino acid sequences in neurofilaments and non-neuronal intermediate filaments

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The major constituents of mammalian neurofilaments are the three triplet proteins of molecular weight 200,000 (200K), 145K and 68K, which also co-migrate in slow axonal transport¹⁻⁵. Partial amino acid sequence data⁶ and chemical cleavage patterns^{7,8} indicate that various cell-specific nonneuronal intermediate filament proteins having molecular weights between 40,000 and 70,000 are structurally related, consistent with a common filament morphology. Here we extend this principle to the neurofilament 68K triplet protein, NF68. We show that NF68, muscle-specific desmin (52K) and mesenchymally derived vimentin (55K) are related proteins. Direct amino acid sequence analysis of a uniquely positioned marker peptide (5K) shows that in this region, NF68 shows ~42% sequence identity with vimentin and desmin, which have ~70% identity. These results are discussed with respect to neurofilament organization.

Treatment of porcine vimentin (55K) and desmin (52K) with the cysteine-specific reagent 2-nitro-5-thiocyanobenzoic acid (NTCB)⁹ yields two fragments (I and II)⁶. Whereas fragments I (37K) spanning the amino-terminal part of the two polypeptides were not further explored, the two carboxy-terminal fragments II (15K) were fully characterized and revealed 64% amino acid sequence identity between vimentin and desmin⁶. Similar treatment of NF68 isolated from porcine spinal cord¹⁰ gave rise to two fragments of 37K (I) and 28K (II) (Fig. 1). End-group analysis localized fragment II to the carboxyterminus (Fig. 2). For direct sequence comparison, we examined cleavage at another amino acid residue, which is rare in intermediate filament proteins^{6-8,11,12}. BNPS-skatole, assumed to

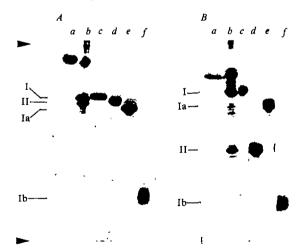


Fig. 1 SDS-polyacrylamide gel electrophoresis of vimentin, desmin and NF68 and their fragments. A and B show NF68 and vimentin, respectively; desmin behaved similarly to vimentin. Porcine proteins (a) were isolated as described elsewhere $^{10-12}$ and cleaved at cysteine $^{6.9}$ to yield fragments I and II (b; see also Fig. 2). The two fragments were isolated by preparative gel electrophoresis⁶ (c and d) and fragments I (c) were further cleaved with BNPS-skatole¹³ to yield two fragments Ia and Ib, which were isolated by preparative gel electrophoresis (e, f). The purified Ib fragments (f) were freed of SDS and processed for protein sequencing as described elsewhere 6.12,26. Alternatively, peptides resulting from cysteine cleavage were mixed and subjected to tryptophan cleavage. Again Ib fragments were isolated from 20% polyacrylamide gels. For localization of fragments see Fig. 2.

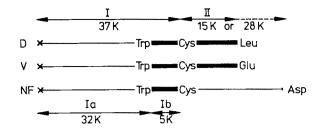


Fig. 2 Organization of the polypeptide chains of porcine desmin (D), vimentin (V) and the neurofilament 68K protein (NF). The relative positions of fragments I and II, obtained by cysteine cleavage of desmin and vimentin, have been given elsewhere⁶. In both cases fragments Ib obtained by tryptophan cleavage occupy the carboxy-terminal part of fragment I, as it has the same carboxyterminal residue (0.8 mol threonine per mol polypeptide by carboxypeptidase Y digestion), whereas fragments Ia contain the blocked (X) amino-terminal end. NF68 fragment II must occupy the carboxy-terminus, as it has the same carboxy-terminal endgroup as NF68 (0.5 mol aspartic acid per mol polypeptide determined by hadrazinolysis), whereas fragment I contains the aminoterminal blocking group and a new carboxy-terminal end (0.3 mol alanine per mol determined by hydrazinolysis). Fragment Ib is assigned again by the end-group results of fragments I, Ia and Ib. Amino-terminal fragments I have approximately the same molecular weights (see Fig. 1), whereas the carboxy-terminal fragments II differ between NF68 and the two other proteins and account for the marked molecular weight differences between NF68 (68K) and vimentin or desmin (55-52K). (For a comparison of the sequences of fragment II from desmin and vimentin, see ref. 6.) Portions of the polypeptides which have been sequenced are indicated by solid boxes.

cleave preferentially at tryptophan¹³, gave a fragmentation pattern indicating a single tryptophan residue situated ~40 residues amino-terminal from the sole cysteine residue documented previously⁶ (Figs 1, 2). Thus double chemical cleavage, first with NTCB and then with BNPS-skatole, provided two new fragments for all three proteins: a large amino-terminal fragment Ia (32K) and a small fragment Ib (5K) in addition to fragment II. Figure 2 indicates that the increased molecular weight of NF68 compared with desmin and vimentin (52-55K) may be accounted for by a larger carboxy-terminal fragment II, the sequence of which has not yet been determined.

The small size of fragments Ib allowed an assessment of the sequence homology suggested by the similar relative localization of tryptophan and cysteine residues in the three distinct proteins (Figs 2, 3). Although of different length (37 and 42 residues), the three fragments are related (Fig. 3). In the case of the vimentin and desmin sequences, 28 out of 39 residues

N Ile - Glu - Ala - Cys

(72%) are identical; this per cent identity is comparable to that found previously for the 140 residues of the corresponding fragments II (64%)⁶. In the case of NF68 fragment Ib, the sequence homology is clearly lower, both in comparison with vimentin (43%) and with desmin (41%) fragments Ib. Thus the similarity in the relative location of cysteine and tryptophan in NF68 compared with vimentin and desmin (Fig. 2) is supported by a similarity in sequence of the resulting fragments Ib (Fig. 3). Although there is a small insertion in this NF68 fragment, many of the other amino acid exchanges are conservative. These results support the hypothesis that neuronal and non-neuronal intermediate filament proteins are related in sequence and belong to a multi-gene family, which is expressed according to certain rules of embryonic differentiation, and which largely coincide with histologically distinct cell types 14-17.

Present models of intermediate filament structure developed for epidermal keratins⁷ and extended to vimentin and desmin⁸ assume a similar 3-polypeptide unit, which contains regions of coiled-coil α -helix interspersed with regions of non- α -helix. Two long α -helices are thought to be present in the aminoterminal two-thirds of the molecules. As it is reasonable to assume that such α -helices have conserved sequences^{7,8} and because our sequence data so far cover only that part of the molecule assumed to be non-helical, more extensive protein data may reveal a higher degree of sequence similarity between different intermediate filament proteins than is presently indicated (our results and ref. 6). Note, however, that in the case of NF68, chain length variation seems to be achieved by an alteration, insertion or addition in the carboxy-terminal domain (Figs 1, 2), whereas previous studies on epidermal keratins indicated that the region between the two helical domains is the major determinant of variant molecular size8.

The sequence homology observed between NF68 and two distinct non-neuronal intermediate filament proteins agrees with our finding that purified 68K protein alone can self-assemble in vitro to form smooth 10-nm filaments¹⁰. What then is the function of the two higher molecular weight neurofilament triplet proteins? They may either act as associated proteins binding to a filament backbone provided solely by the 68K protein, as indicated by antibody decoration studies for the 200K protein present in native neurofilaments^{4,18}, or may themselves form part of the filament backbone. The latter possibility is suggested but not proved by several observations. Although the three triplet proteins possess unique and distinct antigenic determinants^{4,19}, several studies suggest the presence of common determinants^{2,4,17,19-21} defined by a recently characterized hybridoma antibody, which not only recognizes the three triplet proteins but also at least several distinct non-neuronal

Fig. 3 Comparison of the amino acid sequences of the marker peptide Ib (see Figs 1, 2) from porcine desmin (D), vimentin (V) and neurofilament NF68 protein (N). Identical residues in desmin and vimentin are indicated by lines over the desmin sequence. Identical residues in vimentin and NF68K protein are underlined in the NF68 sequence. Note the high degree of homology between desmin and vimentin and the lower homology between these proteins and the NF68 polypeptide. The first (Trp) and last (Cys) residues are not present in the isolated fragments but represent the overlapping residues to fragments Ia and II, respectively (see Fig. 2). They are included in the sequences for better alignment and emphasize the small insertion in the carboxy-terminal region of the NF68 fragment Ib

intermediate filament proteins²². If these results indicate common functional amino acid sequences, the two higher molecular weight triplet proteins may be incorporated at least in part into the filament backbone. This possibility is also suggested by their relative abundance compared with the 68K protein^{1-5,10,17-21,23}, which makes it difficult to envision them solely as associated proteins in the sense of microtubule-associated proteins present in microtubules²⁴. In addition, in the invertebrate Myxicola, neurofilaments have no NF68 and consist of only two polypeptides²⁵ having molecular weights of 150K and 160K, and which are antigenically related to mammalian intermediate filament proteins 17,22. Clearly more chemical and structural information is needed to elucidate neurofilament architecture.

We thank S. Lechte for technical assistance and Dr G. Shaw or discussions. This work was supported by the Max-Planck Society and a grant from the Deutsche Forschungsgemeinschaft (We 338/2).

Note added in proof: We have recently found that bovine glial fibrillary acidic protein (GFA) shows the same distribution of tryptophan and cysteine residues as given in Fig. 2 for desmin and vimentin.

Received 7 January, accepted 13 February 1982

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Influence of ionic current on Na+ channel gating in crayfish giant axon

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In studies of nerve excitation it is important to examine the possible interaction of voltage-sensitive gating mechanisms with components forming the pathway of ionic channels. There is evidence that permeant ions and gating are independent in Na channels^{1,2} of nerve fibres, but not in K⁺ channels³. Of interest in this and other studies of excitability is the determination of the physical basis for the resistance in series with the axon membrane $(R_i)^4$. In an attempt to determine whether an appreciable R, is associated with individual Na⁺ channels⁵, we have compared gating kinetics in conditions in which the ionic current through individual channels is varied while the total membrane current remains constant. The number of conducting Na^+ channels, and thus their contribution to R_* , was lowered by addition of tetrodotoxin6. We report here that no more than 10% of the resistance in 'series' with activation gates is associated with the Na+ channel, and give some preliminary estimates for minimum distances between gates and ion-binding sites within the Na+ channel.

The most common method of measuring R_{\bullet} uses the response to a hyperpolarizing step in current-clamp, a condition in which most Na⁺ channels are closed. The influence of R_* is seen most clearly, however, in a dependence of Na+ channel gating kinetics on the magnitude of Na⁺ currents. As we wished to make no assumptions regarding the identity of these two resistances, our experiments used very small ionic currents and no electronic compensation for R_* . Crayfish giant axons were voltage-clamped and internally perfused^{7,8}. Two external solutions were required: 100% Na contained (mM) Na⁺, 210.2; K⁺, 1; Mg²⁺, 2.6; Ca²⁺, 13.5; Cl⁻, 293.3; HCO₃, 2.3; HEPES, 2.5; 4-aminopyridine, 2, $pH7.70\pm0.05$ at 8°C; 6 % Na was identical except that all but 13.8 mM of Na⁺ was replaced by tetramethylammonium ion. The difference in liquid junction potential at the reference electrode on switching between these solutions was <1 mV and has been ignored. Measurements were made on the kinetics of closing of activation and inactivation gates, first in 6 % Na, then in 100 % Na-3.2 nM tetrodotoxin (TTX). Pulse protocols were then repeated in each solution with 100 nM TTX. Conditions were thus set so that closing kinetics could be measured either with many channels open, but low ionic current per channel, or with few channels open but high current per channel. The experiments were designed to keep total ionic current small and equal in the two cases. After several preliminary experiments to establish the best procedures, the following data were obtained from five axons.

Families of Na⁺ currents used in the determination of inactivation time constants (τ_h) are shown in Fig. 1; τ_h is a measure of the rate of decay of Na+ currents under a maintained depolarization. 100 % Na+3.2 nM TTX provided a good match of peak currents with those in 6 % Na, particularly at lower depolarizations, which were of primary interest. Figure 2 plots τ_h against V_m ; relatively little variation is seen among the different fibres in each solution. The shift in the points from 6 % Na (open symbols) to 100 % Na + 3.2 nM TTX (solid symbols), measured in the region of greatest slope (-20 to -40 mV),

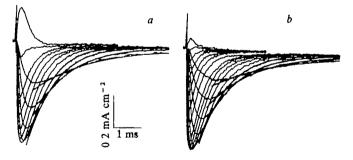


Fig. 1 Families of Na⁺ currents. a, 6% Na; b, 100% Na+3.2 nN TTX. Three consecutive sweeps were averaged by the computer in all cases. K⁺ currents were reduced by 4-aminopyridine^{15,16} and eliminated by subtraction of records in 100 nM TTX. Pulses at low depolarizations were of longer duration than shown to allow an accurate determination of exponential fits to Na+ current decay. Pulses at high depolarizations were of short duration. Fits of single exponentials to inactivation were made using strict and consistent criteria: there was no adjustment of the baseline and the fit was made between 10 and 70% of the peak current. Fits at the four lowest depolarizations are shown superimposed. It has been previously demonstrated 14,17 that inactivation kinetics in the crayfish axon are well described by a single time constant. Holding potential, -76 mV; 50 ms prepulses to -116 mV and a 0.5 ms return to -76 mV preceded depolarizations to a (6 % Na): --31, -26, -21, -16, -11, -6, -1, +4, +14, and +24 mV. In 100 % Na+3.2 nM TTX (b), additional depolarizations to +34, +44, +54, +64 and +74 mV are shown. External solutions are described in the text. The internal perfusate contained (mM) K 230, Na⁺, 5; F⁻, 119; Cl⁻, 5; citrate ion, 37; mannitol, 93; pH 7.35.

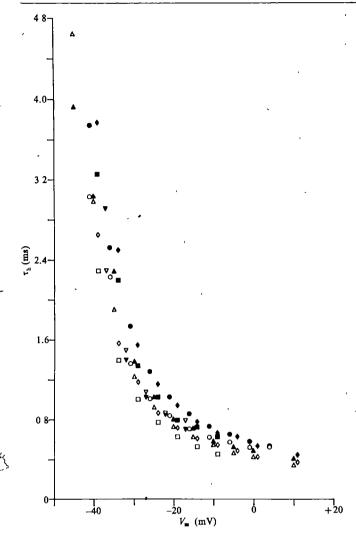


Fig. 2 Inactivation time constants plotted against membrane potential. Data from five axons. Open symbols, 6 % Na; solid symbols, 100 % Na+3.2 nM TTX. Single exponentials were fitted to Na⁺ current decays using a weighted least-squares procedure.

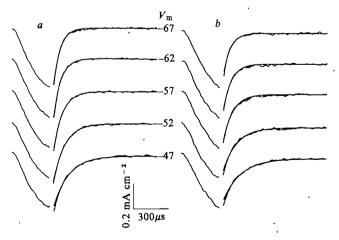


Fig. 3 Na⁺ tail currents. a, 6 % Na; b, 100 % Na+3.2 nM TTX. Three consecutive sweeps were averaged. Holding potential, $-77 \,\mathrm{mV}$. A hyperpolarizing prepulse to $-117 \,\mathrm{mV}$ removed resting inactivation. The fibre was depolarized to $-22 \,\mathrm{mV}$ for 0.4 ms and V_{m} was then changed to the values shown. Records of ionic current are shown after subtraction of traces in 100 nM TTX. Exponential fits are superimposed on tail currents and are generally adequate. At the highest depolarizations used (\sim -45 mV), a second small (<10%) and very slow component ($\tau \simeq \tau_{\mathrm{h}}$) was sometimes seen and was subtracted before fitting. Only 4 of the 44 points used in calculating shifts in Fig. 4 required this procedure.

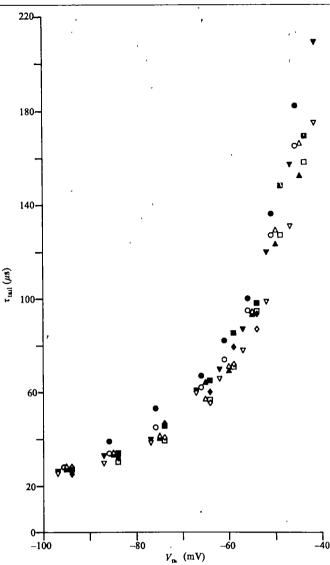


Fig. 4 Na⁺ tail current time constants plotted against membrane potential. Points are from the same axons as in Fig. 2. Open symbols, 6 % Na; solid symbols, 100 % Na+3.2 nM TTX.

is $+3.1\pm1.0\,\mathrm{mV}$ (\pm s.e.m.). This shift is small and is in the opposite direction to that expected for a series resistance. For inward currents, the $R_{\rm e}$ voltage drop causes measured $V_{\rm m}$ values to be more negative than actual membrane potentials.

An example of Na⁺ tail currents is given in Fig. 3. In contrast to $\tau_{\rm h}$, $\tau_{\rm tail}$ measures the rate of decay of Na⁺ conductance when a depolarization is abruptly interrupted and $V_{\rm m}$ returned to a more negative value⁹. Fits to single exponentials are superimposed and are seen to be generally satisfactory. Figure 4 shows $\tau_{\rm tail}$ plotted against $V_{\rm m}$. The shift along the voltage axis from 6 % Na (open symbols) to 100 % Na+3.2 nM TTX (solid symbols), measured between -70 and -45 mV, is -2.0±0.4 mV. This is again small, but this time is consistent with a channel-associated $R_{\rm s}$.

Current-clamp techniques give a total R_{\bullet} of 4–6 Ω -cm² in crayfish axons⁷. Furthermore, with no R_{\bullet} compensation, voltage shifts in τ_h - V_m curves on changing from 100 % Na (no TTX) to 6 % Na, divided by the difference in current amplitude, suggest an R_{\bullet} of 6–7 Ω -cm². We shall use 6 Ω -cm² as a reasonable value in order to estimate the magnitude of some possible sources of error. At midpoints of the voltage ranges used to determine shifts (-30 mV for τ_h and -58 mV for τ_{tail}), average maximum currents were 0.38 mA cm⁻² (τ_{tail}), suggesting that errors due to uncompensated Schwann cell R_{\bullet} should be at most ~2.4 mV. This is confirmed by the observation that despite the significant ranges of maximum currents in different axons in the same solution (for example,

 $0.2-0.5 \text{ mA cm}^{-2} \text{ used } \cdot \text{to measure } \tau_h \text{ in } 6 \% \text{ Na}; 0.3 0.6~\mathrm{mA~cm^{-2}}$ in the case of τ_{tail} in 6 % Na), the time constants vary over only a 2-mV span at these potentials. Our experiments have been designed to reduce such errors further by keeping current magnitudes equal in the two external solutions. The mean difference (value in 6 % Na minus that in 100 % Na+ 3.2 nM TTX) was 0.06 mA cm^{-2} for measuring τ_h and 0.20 mA cm^{-2} for τ_{tail} . $R_s \Delta I$ then represents a small correction so that the shifts along the voltage axis in changing from 6 % Na to 100 % Na + 3.2 nM TTX become +2.7 \pm 1.0 mV for τ_h and -3.2 ± 0.4 mV for $\tau_{\rm tail}$.

We calculate that in the five axons analysed for shifts, the difference in peak Na⁺ currents between 100 % Na (no TTX) and 6 % Na was ~5 mA cm⁻². Since the Na⁺ current per channel should be unchanged by the addition of 3.2 nM TTX¹⁰, the shift in gating kinetics expected if all the R. were associated with individual Na+ channels is -30 mV. Our results suggest that with regard to activation, almost 90% of the R_{\star} is not of this form and is probably a reflection of the Schwann cell connective tissue sheath. Furthermore the small residual resistance shows no systematic voltage dependence in the range of $V_{\rm m}$ in which activation is highly voltage-dependent. Mathias et

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al.5 have postulated that a channel-associated, voltage-dependent R_1 might account for gating charge movement; our results suggest that this is inappropriate for nerve Na⁺ channels. Salzberg et al.11, using an optical probe, measured the Schwann cell contribution to R_{\bullet} in squid axons as 2.8 Ω -cm².

The shifts that were measured in our experiments are small and are in opposite directions. One possible explanation might reside in an electrostatic interaction in which a Na+ ion bound at a site within the channel 12,13 stabilizes the voltage-sensitive components of gates in their open states¹⁴. Because of the opposite voltage dependence of $au_{\rm h}$ and $au_{\rm tail}$ in the $V_{\rm m}$ ranges considered, a slowing of each time constant would result in shifts of opposite sign. From the K_d of 368 mM measured by Hille¹², and the Na⁺ concentration in 100 % Na, at least one site in the channel might be occupied for about one-third of the time the channel is open. Then, for a shift of 3 mV, and assuming a uniform dielectric with no intervening counterions, we calculate the minimum distance between Na+ ion and gate voltage sensor to be 20 Å. If the effective dielectric constant is less than 81, this value would be proportionally higher.

This work was supported by NIH grants 5-R01-NS10500 and R.C.D.A. 5-K04-NS00133.

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Nerve impulses increase the phosphorylation state of protein I in rabbit superior cervical ganglion

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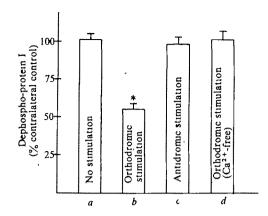
Protein I, a neurone-specific protein concentrated at synapses, is present in most, and probably all, presynaptic nerve terminals where it is at least partially associated with neurotransmitter vesicles (refs 1-5 and P. De Camilli, W. B. Huttner, R. Cameron, M. Harris and P.G., in preparation). Protein I is a major endogenous substrate in nervous tissue for both cyclic AMP-dependent and calcium/calmodulin-dependent protein kinases. Neurotransmitters^{9,10}, apparently acting through cyclic AMP, and depolarizing agents9-11, apparently acting through calcium, have been shown to increase the state of phosphorylation of protein I in intact preparations of the central and peripheral nervous systems. To determine whether impulse conduction regulates the state of phosphorylation of protein I, we have now studied this process in the rabbit superior cervical ganglion, a well characterized neuronal preparation that is suitable for both physiological and biochemical studies. Using a technique developed to quantify the state of phosphorylation of the small amounts of protein I contained in ganglion tissue, we show here that in physiological conditions brief periods of impulse conduction increase the state of phosphorylation of protein I in this ganglion.

New Zealand White rabbits (1.5-2.5 kg) were used in this study. The two superior cervical ganglia of each rabbit contained almost identical amounts of dephospho-protein I when neither ganglion was stimulated (Fig. 1a). Therefore, one ganglion from each rabbit served as the 'test' ganglion and the contralateral ganglion as the control. When the preganglionic nerves supplying the test ganglia were stimulated at 10 Hz for 30 s (a frequency of impulse conduction observed in vivo 12), there was a decrease in the amount of dephospho-protein I compared with that in the contralateral control ganglion (Fig. 1b). As the

total amount of protein I, determined by radioimmunoassay, was not altered by nerve stimulation (data not shown), the results demonstrate that in physiological conditions impulse conduction increased the state of phosphorylation of protein I in the rabbit superior cervical ganglion. In contrast to stimulation of preganglionic nerves (orthodromic impulse conduction), stimulation of postganglionic nerves (antidromic impulse conduction) at 10 Hz for 30 s did not alter protein I phosphorylation (Fig. 1c). Furthermore, the effect of preganglionic nerve stimulation on protein I phosphorylation was dependent on extracellular calcium (compare Fig. 1b with d).

Tables 1 and 2 show the state of phosphorylation of protein I in the rabbit superior cervical ganglion as a function of the number of impulses conducted along the preganglionic nerve. In one series of experiments (Table 1a), the preganglionic nerves supplying test ganglia were stimulated at 10 Hz for various periods of time and the amount of dephospho-protein I present immediately after the period of nerve stimulation was determined. An increase in protein I phosphorylation was first observed after 10 s of nerve stimulation (100 impulses) and appeared to be maximal after 20 s (200 impulses). In a related series of experiments (Table 1b), the preganglionic nerves supplying test ganglia were stimulated at 10 Hz for various periods of time as in Table 1a, and the amount of dephosphoprotein I present 30 s after the initiation of nerve stimulation was determined. In these conditions, 2 s of nerve stimulation (20 impulses) significantly increased protein I phosphorylation and 5 s of nerve stimulation (50 impulses) appeared to produce a maximum in protein I phosphorylation.

In further experiments (Table 2), the preganglionic nerves supplying test ganglia were stimulated for 5 s at various frequencies and the amount of dephospho-protein I present 30 s after the initiation of nerve stimulation was determined. The state of phosphorylation of protein I was increased when ganglia were stimulated at 5 Hz (25 impulses) and 10 Hz (50 impulses), but not at lower frequencies. Figure 2 shows the change in dephospho-protein I observed in response to 5 s of preganglionic nerve stimulation at 10 Hz as a function of the time after initiation of nerve stimulation. An increase in the state of phosphorylation of protein I was first observed 20 s after initiation of nerve stimulation and appeared to be maximal at Fig. 1 Regulation by impulse conduction of the state of phosphorylation of protein I in the rabbit superior cervical ganglion. Rabbits were injected intravenously with 1 ml (50 mg) sodium pentobarbital (Nembutal, Abbott) and then were killed by air embolism. The two superior cervical ganglia of each rabbit, with their pre- and postganglionic nerves, were quickly dissected, placed in a chamber and superfused at room temperature with oxygenated Locke's buffer (mM). 136 NaCl; 5.6 KCl; 20 NaHCO₃, 1 2 NaH₂PO₄; 2 2 CaCl₂, 1 2 MgCl₂, 0.18% glucose (pH 7.4) The ganglia were carefully decapsulated and then superfused for 30-45 min with a-c, standard Locke's buffer; d, calcium-free Locke's buffer (2.2 mM MgCl₂ and 1 mM EGTA were substituted for 2.2 mM CaCl₂) One ganglion from each rabbit served as the 'test' ganglion, and the other as the control a, Test ganglia were mounted in suction electrodes, but not stimulated b, d, The preganglionic nerve supplying the test ganglion was stimulated via a suction electrode with supramaximal pulses at 10 Hz for 30 s. The effectiveness of stimulation was monitored by recording compound action potentials from postganglionic nerves using a second suction electrode c, The postganglionic nerves supplying the test ganglion were stimulated at 10 Hz for 30 s using a suction electrode. Immediately after the 30 s period of nerve stimulation, test and contralateral control ganglia were homogenized in 400 µl of 1% SDS using conical glass tissue homogenizers (Belco). The SDS homogenates were boiled for 5 min and then centrifuged for 2 min in a Beckman microfuge. The supernatants ('SDS extracts') contained essentially all the total protein I as determined by radioimmunolabelling of gels. SDS extracts were used to quantify the amount of dephospho-protein I and the total amount of protein I. The former was assayed by a 'back phosphorylation' technique in which the



protein I in SDS extracts was first immunoprecipitated and then phosphorylated with purified protein kinase. The immunoprecipitation reaction was carried out at 0.4°C in plastic microfuge tubes. Duplicate aliquots (60 µl) of the SDS extract from each ganglion were adjusted by addition of a 200 µl solution to contain (mM; final concentrations) 250 NaC; 50 NaF; 14 EDTA; 10 sodium phosphate, pH 7.4, 15% (v/v) Nonidet P-40. To these were added 4 µl of the γglobulin fraction of anti-protein I rabbit antiserum. The mixture was incubated for 20 min, then 3 µg of purified rabbit IgG (Sigma) and 20 µl of the IgG fraction of goat anti-rabbit IgG (Sigma, 1 ml of goat IgG precipitated 2-3 mg of rabbit IgG) were added. After incubation for 3 h, the mixture was centrifuged in a microfuge for 1 min. The pellet was washed with 200 µl ice-cold 150 mM NaCl/10 mM sodium phosphate, pH 7.4, and then dissolved in 50 µl of ice-cold 11 mM citric acid (final pH ~3). The dissolved pellet was phosphorylated as described elsewhere¹⁰, with the following modifications. The assay mixture (final volume, 70 µl) contained 50 mM HEPES, pH 7.4; 10 mM MgCl₂, 0 1 µM purified catalytic subunit (beef heart cyclic AMP-dependent protein kinase, provided by A. C. Nairn); 15 mM dithiothreitol, 0 005% Nomdet P-40. The phosphorylation reaction was initiated by adding 30 µl [γ-³²P]ATP (final concentration 2-3 µM, specific activity 0 5-1×108° c p.m. nmol⁻¹), and was carried out at 30 °C for 30 min. The reaction was terminated by boiling the mixture in 'SDS-stop solution' as described elsewhere¹⁰ The boiled phosphorylated extract was then subjected to SDS-polyacrylamide gel electrophorories as described previously¹⁰, except that the lower (resolving) gel contained 9% acrylamide with a ratio of acrylamide to methylenebisacrylamide gel electrophorories and described previously¹⁰, except that the lower (resolving) gel contained 9% acrylamide with a ratio of acrylamide to methylenebisacrylamide gel electrophorories as described previously¹⁰, excep

Table 1 Effect of number of impulses of constant frequency (10 Hz) on protein I phosphorylation

а	No. of impulses	Duration of nerve stimulation (s)	Dephospho-protein I immediately after nerve stimulation (% contralateral control)	b	No. of impulses	Duration of nerve stimulation (s)	Dephospho-protein I 30 s after initiation of nerve stimulation (% contralateral control)
	0	0	$102 \pm 4 (6)$		0	0	102 ± 4 (6)
	50	5	$107 \pm 4 (3)$		10	1	$91 \pm 14(3)$
	100	10	$69 \pm 4 (3)*$		20	2	$72 \pm 4(3)^*$
	200	20	$54 \pm 3 (3)^*$		50	5	58± 8 (7)*
	300	30	$56 \pm 4 (7)^*$		· 300	30	$56 \pm 4(7)^*$

The preganglionic nerve supplying one ganglion of each rabbit was stimulated via a suction electrode at 10 Hz for various periods of time. Stimulated and contralateral control ganglia were homogenized in 1% SDS a, immediately after nerve stimulation, and b, 30 s after the initiation of nerve stimulation. Dephospho-protein I was determined as described in Fig. 1 legend, and the amount in the stimulated ganglion was compared with that in the contralateral control ganglion. Data are expressed as mean \pm s e m. Values in parentheses represent the number of pairs of ganglia.

* Significantly different from control (P < 0.05) by two-tailed t-test

Table 2 Effect of number of impulses occurring during a constant interval (5 s) on protein I phosphorylation

No. of impulses	Frequency (Hz)	Dephospho-protein I (% contralateral control)
0	0	$102 \pm 4(6)$
5	1	$101 \pm 15(3)$
10	. 2	$94 \pm 20(3)$
25	. 5	$74 \pm 6 (5)^*$
50	10	58± 8 (7)*

The preganglionic nerve supplying one ganglion of each rabbit was stimulated via a suction electrode for 5 s at various frequencies Stimulated and contralateral control ganglia were homogenized in 1% SDS 30 s after the initiation of nerve stimulation. Dephospho-protein I was determined as described in Fig. 1 legend, and the amount in the stimulated ganglion was compared with that in the contralateral control ganglion. Data are expressed as mean ± s e.m. Values in parentheses represent the number of pairs of ganglia.

* Significantly different from control (P < 0.05) by two-tailed t-test.

30-60 s, after which the state of phosphorylation of protein I returned to control levels.

Related studies (E.J.N. and P.G., in preparation) on the rabbit superior cervical ganglion have indicated that the ganglion contains two 'pools' of protein I. One pool, representing ~60% of total ganglion protein I and which is located in presynaptic nerve terminals, disappears on surgical denervation of the ganglion but is unaffected by brief exposure to the protein synthesis inhibitor, cycloheximide. The other pool, representing ~40% of total ganglion protein I, is located in the cell bodies of the ganglionic neurones. This postsynaptic pool is unaffected by denervation but is decreased by brief exposure to cycloheximide. Thus, postsynaptic protein I appears to represent newly synthesized protein I, presumably en route to the nerve terminals arising from the ganglionic cell bodies.

The state of phosphorylation of protein I is increased not only by impulse conduction, but also by dopamine and by high K^+ in intact rabbit superior cervical ganglia (E.J.N. and P.G., in preparation). In contrast, dopamine and high K^+ did not alter protein I phosphorylation in denervated ganglia. Furthermore, the effect of impulse conduction on the state of

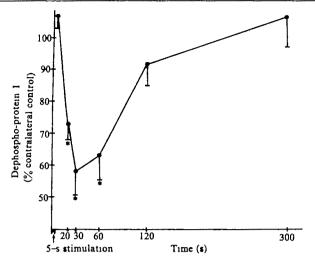


Fig. 2 Effect of a brief period (5 s) of impulse conduction on amount of dephospho-protein I, as a function of time after stimulation. The preganglionic nerve supplying one ganglion of each rabbit was stimulated via a suction electrode at 10 Hz for 5 s; stimulated and contralateral control ganglia were homogenized in 1% SDS at various times afterwards. Dephospho-protein I was determined as described in Fig. 1 legend, and the amount in the stimulated ganglion compared with that in the contralateral control ganglion. Values shown are mean ± s.e.m. The number of pairs of ganglia used ranged from 3 to 7. * Significantly different from control (P < 0.05) by two-tailed t-test.

phosphorylation of protein I was not blocked by those neurotransmitter antagonists that abolish the three postsynaptic potentials observed in the ganglion. These results suggest that the state of phosphorylation of presynaptic protein I, but not that of postsynaptic protein I, is regulated by impulse conduction, by dopamine, and by high K⁺

We have shown that brief periods of impulse conduction at physiological frequencies¹² increase the state of phosphorylation of protein I in the rabbit superior cervical ganglion. This effect is dependent on extracellular calcium, suggesting that impulse conduction increases protein I phosphorylation through the activation of calcium-dependent protein kinase(s)8. We have also found that dopamine and 8-bromo-cyclic AMP increase the state of phosphorylation of protein I in rabbit ganglia, in the presence or absence of extracellular calcium (E.J.N. and P.G., in preparation), suggesting that these agents increase protein I phosphorylation through the activation of cyclic AMPdependent protein kinase1. It is interesting to note in this context that cyclic AMP^{13,14} and calcium^{15,16} have also been shown to modulate neurotransmitter release at various synapses. Catecholamines including dopamine, apparently acting through cyclic AMP¹⁷, and brief periods of impulse conduction, apparently acting through calcium^{18,19}, produce long-lasting facilitation of neurotransmitter release in vertebrate sympathetic ganglia. It is possible that an increase in the state of phosphorylation of protein I may be one of the mechanisms by which certain neurotransmitters and brief periods of impulse conduction facilitate neurotransmitter release.

Received 30 November 1981, accepted 9 February 1982.

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Use of HLA loss mutants to analyse the structure of the human major histocompatibility complex

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Class I loci of the human major histocompatibility complex (MHC) encode 44,000-molecular weight polypeptides that associate nonconvalently with β -microglobulin¹. Included in this category are the HLA-A, -B and $-\bar{C}$ loci (Fig. 1a), which are extensively polymorphic². HLA-specific cDNA clones³. now allow this polymorphism to be studied at the DNA level. However, the lack of sufficient amino acid sequence data for all but a few of the class 1 antigens presents a major difficulty in the allelic assignment of those DNA clones obtained in man³ and mouse⁶⁻⁸. HLA loss variants produced with deletioninducing ionizing radiation offer a means of partially circumventing the time-consuming and difficult task of sequencing HLA class I gene products for identifying restriction enzyme digest fragments of DNA and recombinant DNA clones. P.K. et al.9 used y-ray irradiation followed by immunoselection to obtain many mutants that no longer expressed one or more HLA specificities from the human lymphoblastoid cell line LCL 721. Only the expression of HLA-B8 was lost in one class of mutant, while expressions of HLA-B8 and at least one additional cis-linked HLA allele were lost in mutants of the second class. Some mutants lost expression of the entire haplotype, that is, HLA-A1, -B8 and -DR3 (ref. 9) as well as SB (refs 4, 10 and unpublished results). Recently, anti-HLA-B5 and anti-HLA-A2 sera have been used to isolate mutants that have lost expressions of one or more loci of the haplotype on the other chromosome 6 of LCL 721 (Table 1)10. Data presented here illustrate how one cDNA clone of an HLA class I gene, pHLA-1, in combination with γ -ray-induced HLA loss variants, will be used to identify recombinant DNA HLA clones and to correlate individual DNA restriction fragments with expression of specific HLA alleles.

pHLA-1 (Fig. 1b, c) contains a 513-base pair (bp) cDNA insert which corresponds to the COOH-terminal 46 amino acids of an HLA class I heavy chain plus a portion of the 3'-noncoding region of the mRNA³. To make a preliminary estimate of HLA class I gene family size, genomic DNA was isolated from a heterozygous LCL, 721 (Table 1) and probed with pHLA-1. On hybridization, 721 genomic DNA digested with the restriction enzyme, HindIII, contained 12 distinct bands (Fig. 2a) which ranged in size from 29 to 1.7 kilobases (kb) and differed

At least three factors contribute to the number of bands of genomic DNA that hybridize with pHLA-1 and thereby affect

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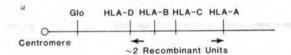
our estimate of the number of class I loci. First, despite the fact that the restriction enzyme used does not cut within the cloned pHLA sequence, it might cut at sites within intervening sequences that separate parts of the pHLA-1 sequence in genomic DNA. Thus, enzymatic treatment could yield two or more fragments derived from a single gene that hybridize with pHLA-1. Second, because the bands which hybridize pHLA-1 differ markedly in intensity, it is possible that the more intense bands represent multiple distinct genes or gene copies. Third, some class I genes may not hybridize with pHLA-1 although this seems unlikely as the amino acid sequence homology¹¹ (88%) between HLA-B7 and HLA-A2 for a significant portion of the heavy chain represented by the coding region of pHLA-1 suggests that the sequence of this region of HLA class I antigens is highly conserved.

Lack of information about the three points above prevents a direct determination of the number of class I genes in the human MHC from the genomic DNA hybridization pattern of pHLA-1. To what extent pHLA-1-positive *Hind*III bands of LCL 721 (Fig. 2a) represent class I loci in addition to *HLA-A*, -B and -C, for example, human analogues¹²⁻¹⁴ of mouse *TL* (ref. 15) and *Qa-2* (ref. 16), or multiple copies of *HLA-A*, -B and -C genes within a haplotype, will require analysis of the corresponding genomic clones. Furthermore, the lack of substantial amino acid sequence information for most *HLA* alleles makes the correlation of genomic clones with allelic specificities exceedingly difficult.

We investigated the usefulness of γ -ray-induced HLA loss mutants for identifying bands in Southern blots with specific HLA loci and their alleles by probing DNA from several such mutants of LCL 721 (Table 1) with pHLA-1. Figure 2 shows the banding pattern found after hybridization of HindIII-digested DNA from six mutants with nick-translated 17 pHLA-1. Four of the mutants had banding patterns that differed from that of the parental LCL 721 cells by either a loss or gain of a band.

Mutant 721.4, a *HLA-B8* loss mutant (Table 1), has every band found in LCL 721 and a new 1.4-kb band not found in LCL 721 that could be explained by a deletion within a larger normal *HindIII* fragment. (Comparison of the digest of 721.4 with those of LCL 721 and 721.18 suggests that possible candidates for the larger band are the 23.5-kb and 1.7-kb bands. These interpretations can be tested by cloning the 1.4-kb fragment and using it to probe the other *HindIII* fragments.)

Simultaneous losses of expression of *HLA-A1*, -B8 and -DR3 are associated with a microscopically visible deletion on



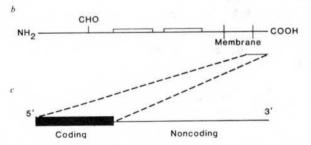


Fig. 1 a, A genetic map of the short arm of human chromosome 6. The genetic markers are: GLO, glyoxalase I. The major histocompatibility loci are HLA-A, HLA-B, HLA-C and HLA-D. b, A schematic depiction of an HLA class I antigen (HLA-A, -B or -C) heavy chain in the plasma membrane. CHO, the carbohydrate side chain and —, disulphide loop. c, Diagram of the pHLA-1 513-bp cDNA insert and its relationship to a HLA class I heavy chain.

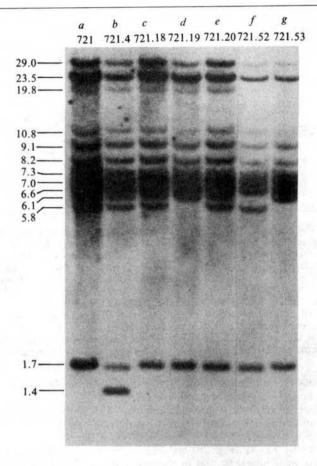


Fig. 2 Southern blot analysis of restriction endonuclease HindIII-digested genomic DNA from LCL 721 and some HLA loss mutants. 15 μg of cellular DNA, isolated as described elsewhere 18, plus 2 μg of λ DNA as a control for digestion, were digested for 18 h with 30 units of HindIII, electrophoresed on a 0.7% agarose, and blotted by the Southern procedure 19. The filter was baked for 2 h at 80 °C in a vacuum oven. Pre-hybridization was in 10 ml of 50 mM NaPO₄ pH 6.5, 50% formamide, 5 × SSC, 5 × Denhardt's 21, 1% glycine and 500 μg ml 3 salmon sperm DNA at 42 °C for 18 h. Hybridization was in 10 ml of 20 mM Na phosphate pH 6.5, 50% formamide, 1 × SSC, 5 × Denhardt's, 100 μg ml salmon sperm DNA, 10% dextran sulphate and 10 c.p.m. of nick-translated 17 pHLA-1 cDNA (specific activity 1.5 × 108 c.p.m. 32 P per μg) for 16 h at 42 °C. The filter was then washed in 1 × SSC for 1 h at 45 °C. Hybridizing bands were detected by autoradiography at -80 °C with a Dupont Lightning-Plus intensifying screen. Sizes of hybridizing bands are in kilobases based on the HindIII fragments of λ. Sources of DNA: Lane a, parental LCL 721; b, 721.4; c, 721.18; d, 721.19; e, 721.20; f, 721.52; g, 721.53. For HLA phenotypes see Table 1.

one chromosome 6 in mutant 721.199. Mutant 721.53, which was derived from 721.19 by γ-ray irradiation and selection for cells that no longer expressed HLA-B5, still expresses HLA-A2 and can be regarded as a HLA-B null mutant. Hybridization of pHLA-1 to HindIII-digested DNA from mutants 721.19 (Fig. 2d) and 721.53 (Fig. 2g) resulted in apparently identical banding patterns. Each mutant lost a 5.8-kb pHLA-1 hybridizing band that is present in LCL 721. Because both mutants have lost only the 5.8-kb band, its absence is correlated with the loss of HLA-B5 expression in 721.53. The 5.8-kb band is present in the HLA-B8 loss variants 721.4, -0.18 and -0.20. Therefore, the absence of the 5.8-kb band is more closely associated with the loss of HLA-A1 expression than of HLA-B8 expression. The other mutant which lost a pHLA-1 hybridizing band was the HLA-A2, -B5, -DR1 loss mutant 721.52 (Fig. 2f). Compared with the 721 banding pattern, 721.52 has lost the 6.1-kb band. The loss of the 5.8-kb band in 721.19 compared with the loss of the 6.1-kb band in 721.52

Table 1 The HLA phenotype of LCL 721 and seven mutants isolated by immunoselection after irradiation of LCL 721 with γ-rays

Cell line	Phenotype					
	DR3	В8	A 1	DR1	В5	A2
Parental						
721	+	+	+	+	+	+
Variants						
4	+	_	+	+	+	+
18	+	_	+	+	+	+
20	+	_	+	+	+	+
19	_	_	_	+	+	+
52	+	+	+	_	_	_
53	_	_	-	+	_	+

The origin of LCL 721 and the procedures for inducing, isolating and characterizing mutants are described in ref. 9. A detailed description of the isolation of mutants 721.52 and 721.53 will appear elsewhere (R. DeM., in preparation). + Refers to expression of marker alleles. 721 and the mother of 721 were typed for HLA-C (C1-C6). Both expressed HLA-C1 only. Mutant 721.52 did not express HLA-C1. This mutant lost expression of the HLA-A2, HLA-B5 haplotype, inherited from the mother, and still expresses HLA-A1 and HLA-B8. We conclude that 721 is heterozygous for HLA-C. HLA-C1 is inherited from the mother on the HLA-A2, HLA-B5 haplotype and something not definable from the father on the HLA-A1, HLA-B8 haplotype.

shows that allelic variations within the HLA complex can be detected by a restriction endonuclease.

Loss of HLA-B5 expression in mutant 721.53 did not result in an additional loss of pHLA-1 hybridizing bands in 721.53 relative to mutant 721.19. Moreover, two of the HLA-B8 loss mutants, 721.18 (Fig. 2c) and 721.20 (Fig. 2e), had banding patterns identical to that of the parental line. The failure to detect alterations in these cases could result from DNA deletions that were too small to detect with our methods or that occurred in HindIII fragments that do not hybridize with pHLA-1. Alternatively, these lines may carry mutations that are not deletions, at least in the coding region of this gene(s). As pHLA-1 corresponds to only the COOH-terminal part of an HLA heavy chain and the 3'-noncoding region of the mRNA, substantial portions of an HLA class I gene could be missed when pHLA-1 is used as the probe. Probing with a nearly full-length HLA cDNA clone⁴ should reveal deletions in the remainder of the gene(s).

Detectable differences in intensity of some bands were also found. As evident from the ethidium bromide staining, some of these are likely to be due to slight differences in the amounts of DNA loaded. For example, more 721 DNA was loaded than from any of the mutants. However, equal amounts of DNA were loaded from mutants 721.18, 721.19 and 721.20 (Fig. 2, c-e). The decreased intensities of the 6.6-10.8-kb bands in 721.19 compared with those in 721.18 and 721.20 are, therefore, consistent with a greater loss of DNA in 721.19 than in 721.18 and 721.20; a complete haplotype in 721.19 compared with a single HLA-B allele in 721.18 and 721.20 (Table 1).

The results described here show the usefulness of using loss mutants in combination with a cDNA probe, pHLA-1, in analysing the organization of the human MHC. They support the concept that HLA class I genes are a multigene family. Similar results have been reported for the H-2 class I gene family in inbred strains of mice^{6,8}. In this regard, note that mutant 721.19 had 11 of 12 pHLA-1 hybridizing bands found in 721. As this mutant has lost one complete HLA haplotype (from HLA-A to HLA-DR), it illustrates that heterozygosity at these loci in 721 has little effect on the number of HindIII bands detected with pHLA-1. The usefulness of radiationinduced HLA loss variants in the analysis of MHC structure is highlighted by the fact that of six variants analysed with one restriction enzyme and a single cDNA probe, three different alterations in the hybridization pattern were detected. Such alterations are not peculiar to HindIII. DNAs from mutants 721.19, 721.52 and 721.53 were also digested with PvuII, PsfI and Sst before probing with pHLA-1. The loss of a pHLA-1 hybridizing fragment was observed with each mutant and all the restriction enzymes (data not shown).

It was conceivable that the pHLA-1 hybridization pattern observed after digestion of DNA with HindIII resulted from reassociation of the 3'-untranslated portion of pHLA-13 with a moderately repeated noncoding DNA sequence. Therefore, a HindIII digest of LCL 721 DNA was probed with a 600-bp fragment containing only the NH2-terminal coding sequence of a nearly full-length HLA cDNA clone⁴. The results show that those pHLA-1-positive bands that are absent in mutants 721.19, 721.52 and 721.53 include the NH₂-terminal sequence (data not shown). Therefore, these bands react with probes for the COOH-terminus and the NH₂-terminus and may contain full-length HLA class I genes. The precise assignment of DNA fragments to specific HLA alleles will require more extensive analyses using newly available mutants, including some that have lost expressions of HLA-A2 alone, HLA-A2 and -B5 or HLA-A1 and -B8, as well as others that are HLA-A-null and HLA-A, -B-null (R. DeM. et al., in preparation).

We acknowledge the help of Mr R. Rudersdorf and Ms C. Chang in the isolation of mutants and the technical help of G. Johnson. This work was supported by NIH research grants AI-15486 (R. DeM. and F.H.B.), AI-17687 (F.H.B.) and AI-10736 (J.L.S.) and by grants from the Leukemia Society of America and the Searle Foundation to H.T.O.

Received 21 September 1981, accepted 26 January 1982

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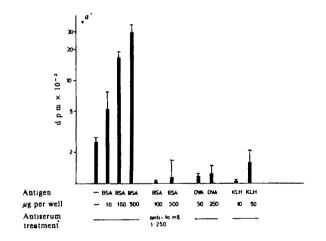
A cloned T-cell line from a tolerant mouse represents a novel antigen-specific suppressor cell type

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Two major alternative models, clonal deletion and suppression, have been proposed as possible explanations for immunological tolerance^{1,2}. We have now isolated a functional T suppressor (T_s) cell clone to analyse the role of T_s cells in the induction and maintenance of tolerance. This cloned, long-term cultured T, cell line (HF1) was derived from CBA/J mice $(H-2^k)$ in which low-zone tolerance (LZT)3 had been induced by subimmunogenic doses of bovine serum albumin (BSA). HF1 cells show BSA-specific suppressor activity in two *in vitro* suppressor assays. HF1 T_a cells and a factor derived from them suppress unprimed, carrier-specific T helper (T_k) cells in an assay measuring IgG antibody production against fluorescein-conjugated BSA (Flu-BSA). Furthermore, they block DNA synthesis in an antigen-specific *in vitro* T-cell proliferation assay. Their proliferation in the presence of antigen-presenting cells is antigen specific and shows restriction to the I-A^k region of the major histocompatibility complex (MHC). In addition, HF1 T_a cells have a characteristic cytotoxic activity. The data reported suggest that clonal deletion and suppression models are not necessarily mutually exclusive. Clonal deletion could manifest itself by the action of T_a cells.

LZT to the antigens BSA and phage fd is accompanied and maintained by T_s cells⁴⁻⁶. Genetic control of LZT induction is, in at least one case, linked to the Igh-1 allotype⁷. To analyse cellular interactions at the molecular level in tolerant animals, it is necessary to produce T-cell lines with specific immunological functions. In an attempt to produce an antigen-specific T_s cell line with the functional properties of T_s cells from low-zone-tolerant mice, CBA/J mice were tolerized by seven consecutive injections of 10 µg BSA per mouse. We anticipated that this sub-immunogenic dose of BSA would activate T_s cells but not T_h cells⁴, and such tolerogenic treatment should therefore preselect T_s cells in vivo and endow them with the capacity to proliferate preferentially on subsequent in vitro selection. After



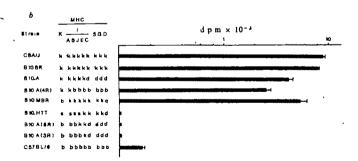


Fig. 1 Antigen specificity and I-A^k restriction in the proliferation of HF1 T_a cells. a, 10⁴ HF1 T_a cells were cultured in 250 μl of DME medium supplemented with 0.5% mouse serum in the presence of 5×10³ 3,000 R measured by addition of BSA or ovalbumm (OVA) and keyhole limpet haemocyanin (KLH) as control antigens. In some experiments BSA-specific proliferation of HF1 T_a cells was inhibited by adding 1 μl monoclonal anti-Ia.m6 antibodies per well. ³H-TdR uptake was measured on day 4 of culture as described in Table 2 legend. b, 10⁴ HF1 T_a cells were cultured in 200 μl DME medium supplemented with 10% FCS and 10% CM in the presence of 5×10⁵ 3,000 R irradiated spleen cells from various congeneic mouse strains, allowing mapping of I-A^k restriction. Proliferation of HF1 T_a cells was measured by ³H-TdR uptake on day 5 of culture as described in Table 2 legend.

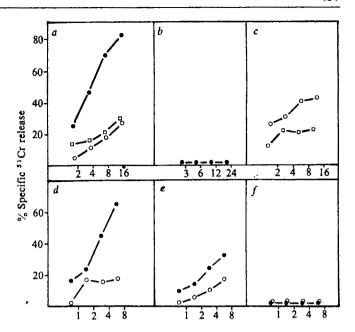


Fig. 2 Cytotoxicity of clone HF1 and subclone HF1.17 T_a ceils against various target cells. Cytotoxicity was measured in a 6-h ⁵¹Cr-release assay using 2×10⁴ target cells per microtitre plate well. Per cent specific ⁵ release is plotted on the ordinate against the effector/target cell ratios on the abscissa. $a_1 \times 10^7$ CBA spleen cells per ml were stimulated with 5 µg mi⁻¹ Con A in DME medium with 10% FCS for 2 days. Cells were washed once in balanced salt solution containing 10 μg mi⁻¹ α -methylmannoside and cultured 1 day longer in DME medium with 10% FCS in the presence of 5% CM. Lymphoblasts were purified on a Fleoil gradient, then labelled with ⁵¹Cr in the form of sodium chromate and used as target cells. HF1

T, cells were tested in the absence (O) or presence (•) of 10 µg mi⁻¹ Con A. Target cell killing is observed in the absence of Con A but can be enhanced in the presence of Con A. HF1.17 cells were tested in the absence of Con A (

). b, C57Bl/6 EL4 T-lymphoma cells were used as targets. HF1 T_a cells cannot lyse these targets even in the presence (●) of Con A. This is an important finding because clone HF1 does not behave like Ta cells, which type target cells nonspecifically in the presence of Con A. c, 2×10^7 CBA spleen cells per ml were cultured for 3 days in DME medium with 10% FCS in the presence of 40 µg ml⁻¹ LPS. Lymphoblasts were purified on a Ficoll gradient and used as target cells. Killing by HF1 (O) and HF1.17 ([]) T, cells is shown. Target cells used in the killing by HF1.17 T_a cells were further purified after Ficoll gradient separation by incubation on anti-Thy-1.2-coated Petri dishes. Non-adherent cells (>98% Ig⁺) were used in the ⁵¹Cr-release assay. d, BSA-specific long-term cultured T cells (LNC-BSA) were derived from lymph node cells of BSA-primed CBA/J mice. They were used as target cells and assayed for killing by HF1 T, cells in the absence (O) or presence (O) of Con A. e, A BSA-specific T-cell clone (LNC-BSA.D.10) derived from LNC-BSA cells was used as target. Assay was in the absence (O) or presence (I) of Con A. f, A HGG-specific long-term cultured T-cell line (LNC-HGG) was used as target cells in the absence (O) or presence (O) of Con A. Lysis of LNC-BSA (e) but not of LNC-HGG (f) cells by clone HF1 cells suggests antigen specificity in killing.

the last in vivo antigenic treatment, splenic T cells were enriched by removing B cells on antibody-coated Petri dishes⁸. They were stimulated with antigen in bulk culture for 4 days and then cloned in soft agar⁹. HF1 cells were derived from 1 out of more than 100 colonies growing, and expanded. We have so far avoided virus transformation¹⁰ or T-cell hybridoma production¹¹ so as to study cellular functions both in terms of cellular interactions and factor production. The following surface characteristics were assessed in indirect immunofluorescence staining tests using fluorescein-conjugated rabbit antimouse or anti-rat immunoglobulin antibodies (Nordic): Thy-1.2⁺ (as defined by an AKR anti-CBA antiserum; Searle); Lyt-1⁻, 2⁺ (as defined by monoclonal antibodies, clones 53-7.3 and 53-6.7; Becton Dickinson); I-A^{k+} and I-E^{k+} (using monoclonal antibodies against Ia.m6, clone H 116-32.R5, and against Ia.m7, clone 13/4.R5; Camon). No I-J^k determinants could be detected by functionally active anti-I-J^k sera¹² from B10.A(3R) mice immunized with B10.A.(5R) lymph node and spleen cells.

Table 1 Suppressor activity of long-term cultured, soft agar-cloned HF1 T, cells and a factor derived from them in an in vitro suppressor assay

	,	Anti-Flu response (PFC per 10 ⁶ cultured cells)		
Cells in culture	Antigen in culture	IgM	IgG	
8×10 ⁵ Flu-HGG primed SC+1×10 ⁵ HF1 8×10 ⁵ Flu-HGG primed SC+1×10 ⁵ 4CBA-C 8×10 ⁵ Flu-HGG primed SC+1×10 ⁵ HF1	Flu-BSA Flu-BSA Flu-HGG	377±14 271±25 904±16	79 ± 23 598 ± 27 $1,339\pm124$	
8×10 ⁶ Flu-HGG primed SC+1×10 ⁵ 4CBA-C 8×10 ⁶ Flu-HGG primed SC+1×10 ⁵ HF1.17 8×10 ⁶ Flu-HGG primed SC+1×10 ⁵ normal SC	Flu-HGG Flu-BSA Flu-BSA	810±14 —	$1,810\pm80$ 67 ± 21 301 ± 21	
8×10 ⁶ Flu-HGG primed SC+1×10 ⁵ HF1.17 8×10 ⁶ Flu-HGG primed SC+1×10 ⁵ normal SC	Flu-RRBC Flu-RRBC	—, —, —	1,144±64 837±44	
7×10 ⁶ Flu-HGG primed SC+1×10 ⁶ BSA-LZT SC 7×10 ⁶ Flu-HGG primed SC+1×10 ⁶ normal SC 7×10 ⁶ Flu-HGG primed SC+1×10 ⁶ BSA-LZT SC	Flu-BSA Flu-BSA Flu-HGG	. 408±10 426±6 349±12	<5 439±29 3,047±63	
7×10 ⁶ Flu-HGG primed SC+1×10 ⁶ normal SC 8×10 ⁶ Normal SC 8×10 ⁶ Normal SC	· Flu-HGG Flu-BSA Flu-HGG	641±16 <5 16±3	2,675±79 <5 <5	
8×10 ⁶ Flu-HGG primed SC+1×10 ⁵ HF1 8×10 ⁶ Flu-HGG primed SC+HF1 supernatant* 8×10 ⁶ Flu-HGG primed SC	Flu-BSA Flu-BSA Flu-BSA	_ _ _	27±20 33±9 250±29	
8×10 ⁶ Flu-HGG primed SC+HF1.17 supernatant 8×10 ⁶ Flu-HGG primed SC	Flu-BSA Flu-BSA	_	69 ± 37 182 ± 15	

T, line HF1 was produced in March 1980 in the following way. For induction of LZT to BSA, CBA/J mice received 2.5 mg hydrocortsone followed by seven consecutive injections of 10 μg BSA per mouse intraperitoneally (1,p.)². One day after the last tolerogenic dose, splenic cells were incubated on Petri dishes coated with rabbit anti-mouse immunoglobulin antibodies. The non-adherent T cells were removed and cultured in Costar plates (1.5×10⁶ cells per well) in the presence of syngencic 3,000 R irradiated spleen cells (5×10⁵ cells per well) in a final volume of 1 ml Dulbecco's modified Eagle's (DME) medium supplemented with 5×10⁻⁵ M 2-mercaptoethanol, 2 mM glutamine, 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin (DME medium) and 10% heat-inactivated horse serum (HS). At the beginning of culture, 10 μg BSA per ml were added to restimulate presumptive T, cells in vitro. Four days later growing cells were cloned in soft agar⁹. On day 3 the agar was overlayered with 250 μl of a supernatant from Con A-atimulated rat spleen cells (conditioned medium, GM)²⁰ One to two weeks after starting the cloning procedure, colonies containing 40–100 cells were transferred to flat-bottomed microtitre wells and cultured in the presence of antigen (10 μg BSA per ml), 3×10⁴ syngeneic 3,000 R irradiated spleen cells and 10% CM in the same medium as described above. Cell cultures were fed every 3–4 days with antigen, 3,000 R irradiated syngeneic squence cells and CM. After 4–6 weeks of continuous cell growth, proliferating cells were expanded on Costar plates or in Falcon 3013 tissue culture flasis. Instead of 10% HS and 10 μg ml⁻¹ BSA, 10% heat-inactivated fetal calf serum (FCS) (containing BSA as antigen) was now used. HF1 cells can be frozen in liquid N₂ and thawed with recovery of growth and function. Suppressor activity of HF1 cells was tested in an *in vitro* assay with Flu-BSA as antigen. This assay has been developed as to have an IgG *in vitro* response which depends on primary T_b-cell activation: CBA/J m

* Equivalent to 1×10⁵ cells.

Table 2 Suppressive activity of HF1 T, cells in an antigen-specific in vitro
T-cell proliferation assay

Lymph node	Antigen or		Incorporation
cells in	Con A in		of ³ H-thymidine
culture	culture	Addition of	(d.p.m.)
Expt 1			
BSA primed	austra .	-	$8,953 \pm 3,838$
BSA primed	25 μg cBSA	_	$51,120 \pm 2,291$
BSA primed	25 μg cBSA	3×10 ⁴ HF1 cells	$12,963 \pm 2,697$
BSA primed	100 ug HGG	_	14.806 ± 6.268
HGG primed		 `	4.578 ± 1.305
HGG primed	100 ug HGG		16.466 ± 917
HGG primed	100 µg HGG	3×10^5 HF1 cells	$15,892 \pm 913$
Expt 2			
BSA primed		_	9.615 ± 1.606
BSA primed	500 mg BSA		54,367 ± 4,120
BSA primed	500 µg BSA	2×10 ⁴ HF1 cells	17.818 ± 2.236
BSA primed	500 μg BSA	Antı-Ia.m6 1:250	14.541 ± 1.952
BSA primed	0.6 μg Con A	_	77.350 ± 2.244
BSA primed	0.6 µg Con A	Anti-Ia.m6 1:250	$99,044 \pm 2,281$

The T-cell proliferation assay using inguinal lymph node cells from antigenprimed CBA/J mice was used as described elsewhere 13, 2×105 lymph node cells were cultured in flat-bottomed microtitre wells with 3×10⁵ 3,000 R ırradiated syngeneic spleen cells in 250 μl of DME medium containing 0.5% mouse serum. At the beginning of culture they were stimulated with either 25 ug heat-aggregated BSA (cBSA) or 100 µg HGG (expt 1) and 500 µg BSA or 0.6 µg Con A (expt 2) per well. Antigen-specific suppressor activity of HF1 cells was determined by addition of 2×10⁴ or 3×10⁴ cells per well. The same degree of suppression was also demonstrated if HF1 cells were treated with mitomycin C before addition to culture (data not shown). Monocional anti-Ia m6 antibodies (1 µl per well) were added to demonstrate its influence on BSA-specific T-cell proliferation. Proliferation was measured on day 4 by pulsing the culture with 0.4 μCl ³Hthymidine (3H-TdR) for 16 h and measuring 3H-TdR uptake. For each experimental condition three independent cultures were set up and mean d.p.m. ±s.d. are given. Lymph node cells were taken from CBA/J mice immunized with 1 mg BSA or 1 mg HGG per mouse in CFA subcutaneously at the base of the tail. Animals were not used until 10 days after immunization.

HF1 cells are Ig⁻ (direct immunofluorescence staining with fluorescein-labelled rabbit anti-mouse Ig antibodies) and FcR⁻(binding of aggregated fluoresceinated human globulin, Flu-HGG).

HF1 T_s cells are functionally active, as demonstrated by suppression of IgG but not IgM anti-Flu antibody production after stimulation with Flu-BSA in an in vitro suppressor assay using unprimed T_h cells and Flu-primed B cells. This suppression is comparable with the effect of BSA-specific T, cells from LZT mice (Table 1). In an independent assay, HF1 cells suppressed antigen-specific BSA-induced in vitro proliferation of lymph node cells¹³ from BSA-primed CBA/J mice. This antigen-specific proliferation was also inhibited by monoclonal anti-I-Ak antibodies in place of HF1 Te cells, indicating that stimulation is I-Ak restricted (Table 2). This BSA-induced in vitro T-cell proliferation assay was used to analyse subclones of HF1 T, cells. HF1 T, cells were subcloned by limiting dilution microculture (1 and 0.3 cells per microplate well) in the same culture conditions as described in Fig. 1b legend. Plating efficiencies ranged from 70 to 100%. All 22 subclones expanded and tested for functional activity showed suppressive activity (data not shown), indicating that HF1 T, cells represent a homogeneous population.

Restriction analysis was performed to identify genes of the H-2 region which regulated proliferation of HF1 T_s cells in the presence of antigen. We first established that the proliferation of HF1 T_s cells requires antigen in the presence of irradiated syngeneic stimulator cells (Fig. 1). This proliferation can be inhibited by supplementing the cultures with monoclonal anti-I-A^k antibodies. Mapping experiments show that their proliferative response is restricted to I-A^k recognition in the MHC. An influence of the I-J^k region is excluded (Fig. 1).

I-A' determinants on HF1 T, cells can be labelled biosynthetically using ³⁵S-methionine (unpublished data), showing that they are synthesized by HF1 T, cells themselves. These data might be a key to understanding differences in the antigendependent activation processes of T_h and T_e cells. Expression of I-A determinants on cloned T_h cells has not been found, but available evidence from heterogeneous Th-cell populations suggests that there is little expression of I-A determinants on these cells.¹⁴ One could envisage a competitive intervention of T_e cells in the interaction between antigen-presenting, I-A determinant-bearing macrophages and T_h cells. The suppressor effect of HF1 T, cells can also be demonstrated using cell-free supernatants from HF1 T, cells. The factor both suppresses IgG anti-Flu antibody production in vitro on challenge with Flu-BSA (Table 1) and inhibits BSA-specific T-cell proliferation (data not shown). The factor has not been analysed in detail and it remains to be seen whether it is different from the suppressor factor produced by a T_s-cell clone from hyperimmune mice15

The additional cytotoxic activity of HF1 T, cells (Fig. 2) distinguishes them from all other T_s-cell lines described so far. HF1 T_s cells can kill concanavalin A (Con A)- or lipopolyssacharide (LPS)-stimulated syngeneic spleen cells but not allogeneic target cells, even in the presence of Con A. Cytotoxic T (T_c) cells nonspecifically lyse target cells in the presence of Con A, due to cell-cell bridging^{16,17}. This implies that the mechanism of cytolysis through HF1 T, cells is different from T_c cell activity.

Subclone HF1.17 isolated from microcultures seeded with an average of 0.3 HF1 cells per well has cytotoxic activity (Fig. 2) and produces a suppressive factor (Table 1). As we were unable to demonstrate cytolysis by the factor itself, a cloned cell of the HF1 T, line must possess two functional properties, factor-mediated suppression and cell-mediated cytolysis.

In considering implications of the above findings for possible mechanisms of immunological tolerance, one must be aware that no definite proof has been found for the absence of T, cells in cases of apparent clonal deletion 18. A presumed incompatibility of the extremes of suppression and deletion models is conceptually largely based on the assumption that clonal deletion is due to a direct interaction of antigen with the cell to be rendered tolerant19. Our finding of a novel type of T, cell clone provides an experimental basis for a combination of both models, suggesting that clonal deletion could be a consequence of T, activity.

We thank Ms P. Wippern for technical assistance and Mrs H. Storkebaum for preparing the manuscript. The work was supported by DFG grants Ko 379/9-10.

Received 21 December 1981, accepted 22 February 1982

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Mouse spleen and IgD-secreting plasmacytomas contain multiple IgD δ chain RNAs

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Immunoglobulin gene expression requires that cells combine the correct genetic information by DNA rearrangement and RNA processing to produce mature, translatable mRNAs. The mechanisms that operate to produce IgD heavy chain (δ chain) mRNA are thought to be especially complex1-3. Like other immunoglobulins, IgD exists in membrane and secreted forms^{4,5}. By analogy with results of studies of μ^{6-10} and $\gamma^{11,12}$ gene expression, we expected that δ chains would also be encoded by two different mRNAs, containing common sequence information transcribed from constant region gene segments but different information transcribed from coding sequences located 3' to the constant region gene segments. Instead, we identified¹³ more than two δ mRNAs in normal mouse spleen and in two IgD-secreting plasmacytomas¹⁴, TEPC 1017 and TEPC 1033. Now we have used 32P-labelled DNA fragments prepared from a δ cDNA clone¹⁸ and a δ genomic clone ¹⁶ to characterize by hybridization these δ RNAs, fractionated on methyl mercury hydroxide agarose gels. We find that normal mouse spleen contains a major 2.9-kilobase (kb) and a minor 2.1-kb RNA encoding membrane-bound δ chains (δ_m) and that TEPC 1017 and TEPC 1033 contain similar $\delta_{\rm m}$ RNAs plus a 1.75-kb RNA encoding secreted δ chains (δ_s). We have also observed less abundant δ RNA species (2.65 and 3.2 kb). Different gene segments or combinations of segments are used in the 3' termini of the multiple δ RNAs.

The δ chain synthesized by normal spleen cells bearing IgD on their surface is presumed to be derived from a complex transcription unit, including a rearranged μ gene^{1-3,17}. In contrast, synthesis of δ chain for secreted IgD is postulated to require deletion of the μ gene^{1-3,17}. Figure 1 shows that TEPC 1017 contains most of its constant domains on a 3.3-kilobase pair (kbp) HindIII fragment, which is smaller than that of liver (4.0 kbp), and that TEPC 1033 contains its δ on a larger (4.6 kbp) fragment. These data are compatible with the relocation of different V-D-J complexes 5' to the C81 domain, between the HindIII site 2.0 kbp 5' to Co1 and the EcoRI site

0.5 kbp 5' to Cδ1 in these plasmacytomas¹⁶

DNA rearrangements 3' to expressed constant region genes have also been reported in plasmacytomas¹⁸, though only rarely. Figure 1 demonstrates that $C\delta$ genes in liver and both plasmacytomas are contained in a single 9.8-kbp EcoRI fragment. This fragment, which was isolated from BALB/c liver and cloned, has previously been shown to contain the C δ gene segments 16 plus virtually all the 3' gene segments to be discussed here 17. Thus, no detectable rearrangements 3' to the δ constant domains have occurred in these tumours, although the possibility of small deletions or rearrangements within the 9.8-kbp fragment cannot be excluded. We assume that (1) this portion of the DNA is identical in liver, the IgD-secreting plasmacytomas and IgD-bearing cells in normal spleen; and (2)

all the δ RNAs we observe in the plasmacytomas and in normal spleen are derived from δ gene transcription units identical in their 3' sequence to that found in liver DNA

The 1.75-kb δ RNA identified in TEPC 1017 and TEPC 1033 RNA is the most abundant δ RNA in these plasmacytomas and is not detected in normal spleen RNA (Fig. 2a). Both plasmacytomas secrete large amounts of IgD protein14; spleen cells bear IgD on their surfaces but fewer than 1 in 105 cells secrete IgD19. Thus, it is likely that the 1.75-kb RNA species is mRNA encoding δ chain for secreted IgD and that one or more of the less abundant δ RNA species in plasmacytomas are mRNAs encoding δ chain for membrane-bound IgD.

The hybridization data presented in Fig. 2 and summarized in Fig. 3 show that the only gene segment to the 3' side of $C\delta 3$ represented in the 1.75-kb δ RNA is contained in the DC1 fragment. DNA sequence data1 reveal that the exon contained in the DC1 fragment encodes a non-hydrophobic polypeptide consistent with the carboxy-terminal sequence of a secreted form of heavy chain.

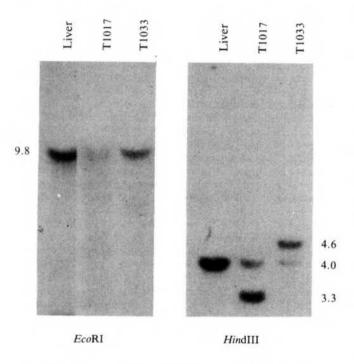


Fig. 1 Cδ hybridization analysis of EcoRI- and HindIII-digested genomic DNA from BALB/c mouse liver and TEPC 1017 and TEPC 1033 tumours. Fragments generated by digestion of 25 µg of each DNA were fractionated by electrophoresis in 0.7% agarose gels, denatured in alkali, neutralized and transferred to a sheet of nitrocellulose²⁴. The sheet was hybridized to a 563-bp DNA fragment (containing only $C\delta$ sequences) that had been excised from p δ 54J plasmid¹⁵ by digestion with PvuII and HindIII, purified by electrophoresis on a 4% polyacrylamide gel²⁵ and labelled with ³²P by nick-translation²⁶ to 1.0×10^8 c.p.m. μg^{-1} of which 2.5×10^6 c.p.m. were used in 25 ml hybridization solution. Lengths of hybridizing fragments (in kbp) were determined by comparison with ethidium bromide-stained EcoRI and HindIII fragments of phage & DNA and are indicated. The pattern of δ-hybridizing fragments in liver DNA is consistent with the previously published restriction map of the δ gene cloned from BALB/c liver 16; immunoglobulin genes in liver DNA are assumed to remain in germ-line configuration. Both tumours have ~25% of their δ DNA in the form seen in liver as determined by densitometric scanning of the autoradiogram. Both plasmacytomas are pseudotetraploid (G. Klein and S. Ohno, personal communication), and it seems likely that one of the four chromosomes remains unrearranged while the other three have undergone rearrangement. However, because the plasmacytoma DNAs were prepared from solid tumours, it is possible that contaminating normal tissue contributed some or all of the unrearranged genes seen in these digests.

The only other δ RNA species that hybridizes with DC1 is the much less abundant 3.2-kb δ RNA in TEPC 1017 and TEPC 1033 (Fig. 2c). This RNA also hybridizes with the AC1 probe (Fig. 2b), and it may represent a short-lived precursor of the 1.75-kb δ_s mRNA.

Membrane IgD is present on most splenic B cells20 and on TEPC 1017 and TEPC 1033 (ref. 14). We presumed that δ_m mRNA would be the most abundant δ RNA in spleen as well as being readily detectable in RNA from these plasmacytomas. The 2.9-kb δ RNA meets these criteria (Fig. 2a) and probably encodes δ chain for membrane IgD. This 2.9-kb δ_m mRNA hybridizes with VDC1 (Fig. 2e) and VDC2 (Fig. 2f) but not with DC1 (Fig. 2c). The DNA sequence 17 of VDC1 encodes a hydrophobic polypeptide ~50% homologous to the amino acid sequence of the δ_m transmembrane polypeptide. This is further evidence that the 2.9-kb δ RNA is a form of δ_m mRNA.

A less abundant δ RNA (2.1-kb) in the plasmacytomas and in spleen also hybridizes with VDC1 sequences. It probably represents another form of δ_m mRNA lacking sequences encoded in VDC2 (Fig. 2f) or VDC3 (data not shown) which are found in the 2.9-kb δ_m mRNA.

Whereas the 2.9-kb δ RNA in the plasmacytomas hybridizes with AC1 (Fig. 2b), the 2.9-kb δ RNA from spleen does not. Thus, plasmacytoma cells may contain at least two δ RNAs in the 2.9-kb band: one (containing AC1 sequences) may be a precursor of the 2.1-kb δ_m mRNA, and another (similar to that in spleen) may be an alternative form of δ_m RNA containing a different 3'-untranslated region.

The 3.2-kb δ RNA species is rare in both spleen and plasmacytomas. As discussed above, in the plasmacytomas it appears to be a form of δ_s RNA. The 3.2-kb δ RNA in the spleen hybridizes with VDC1, VDC2 and VDC3 (Fig. 2e, f) but not with DC1 (Fig. 2c), suggesting that it is a form of δ_m RNA, either a third mature form of δ_m mRNA in the spleen, or a precursor of one of the other two δ_m mRNAs.

The DC2 probe hybridizes (Fig. 2d) with a 2.65-kb RNA species but with none of the other δ RNAs described here. The amount of the 2.65-kb RNA species varies among different preparations of plasmacytoma and spleen RNA and is by far the least abundant δ RNA detected. Thus the composition of this δ RNA with respect to other DC, AC or VDC sequences

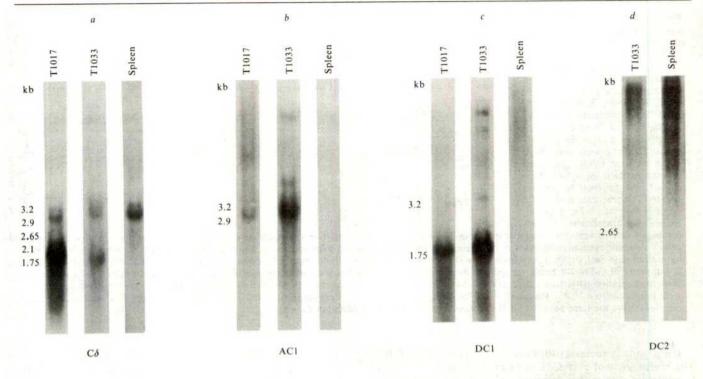
and its possible function cannot yet be stated.

Our studies of normal spleen and two IgD-secreting plasmacytomas suggest that δ chain is unusual among immunoglobulins in that its mRNAs exist in so many forms. While some of these δ RNAs may represent aberrant transcripts analogous to the 'C, RNAs' described by others21,22, their presence in normal spleen argues against their being abnormal. Preliminary experiments have identified the 2.1-, 2.9- and 3.2-kb δ RNAs on polyribosomes prepared from B-cell lymphomas which also indicates that all three are normal, functional mRNAs. The complexity of the δ gene transcripts contrasts with the mouse IgM8,9 and IgG11,12 heavy chains, each of which is encoded by only two mRNAs, one for the membrane form and one for the secreted form of the protein.

Other groups^{2,3} have studied δ RNA in B-cell tumours and hybridomas, but none has reported the complex array of δ RNAs identified here in normal spleen and plasmacytomas. The 1.8-kb δ RNA reported by these groups is probably identical to the δ_s mRNA we report. Similarly, their 2.7-kb mRNAs^{2,3} may be identical to the 2.9-kb δ_m mRNAs we report here. The less abundant RNAs reported here of 2.65 kb and 3.2 kb have not been described by other groups, although Maki et al.3 noted that R-loops observed but not presented suggested

that there could be other forms of δ RNA.

We have identified δ RNAs in spleen and TEPC 1017 and TEPC 1033 which encode the membrane form(s) of δ chain and share certain significant characteristics. Most important, a hydrophobic (transmembrane) polypeptide17 is encoded in both the 2.9- and the 2.1-kb δ_m mRNAs found in spleen and the plasmacytomas. In addition, the 2.9-kb δ_m mRNAs from both sources contain sequences encoded in VDC2 and VDC3.



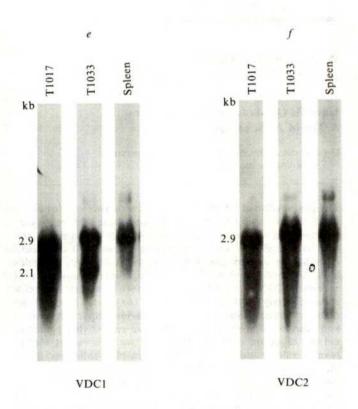


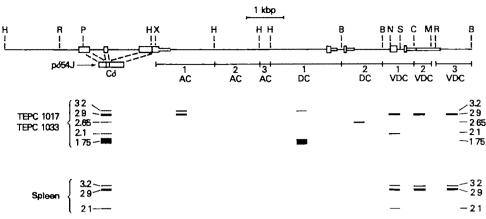
Fig. 2 Hybridization analysis of poly(A)+ RNAs prepared from IgD-secreting plasmacytomas and normal spleen. Cytoplasmic RNA was prepared as described previously¹⁵ from TEPC 1033 ascites cells which had been maintained in tissue culture. Total cellular RNA was prepared by homogenizing pulverized frozen plasmacytoma tumours or BALB/c spleens in 4 M guanidinium thiocyanate and precipitating with ethanol²⁷. Total cellular RNA also was prepared from spleen by a LiCl-urea extraction procedure²⁸. Poly(A)⁺ RNA was prepared by selection on oligo(dT)cellulose and treated with methyl mercury hydroxide before being fractionated on 1% (or 0.7% in the case of b and TEPC 1017 lanes in e and f) agarose gels containing 5.0 mM methyl mercury hydroxide²⁹. RNA was leaded at 5 μ g per lane. TEPC 1033 poly(A)+ RNAs in b and c were prepared from total cytoplasmic RNA; spleen poly(A)+ RNA in d-f was prepared from total cellular RNA isolated by the LiCl-urea extraction procedure. All other poly(A)+ RNAs shown were derived from total cellular RNAs prepared by the guanidinium thiocyanate extraction procedure. Following electrophoresis, the RNA transfer and hybridiz-ation was carried out as described elsewhere 30,31 except that DPT was used instead of diazobenzyloxymethyl paper. The restriction fragments (see Fig. 3) used for nick-translation were isolated from polyacrylamide gels²⁵. The specific activities of the 32 P-labelled probes ranged from 2×10^7 to 4×10^8 c.p.m. μg^{-1} ; 106 c.p.m. ml⁻¹ of hybridization mixture were used. The probes described in Fig. 3 were each hybridized to a set of lanes containing the RNA from TEPC 1017, TEPC 1033 and spleen cells (a, Cδ; b, AC1; c, DC1; d, DC2 (which required an unusually long exposure time); e, VDC1; f, VDC2). The approximate sizes of the hybridizing species (in kb) discussed here are shown to the left of each panel and were determined by reference to ethidium bromide-stained mouse 18S and 28S rRNAs which were assumed to be 2.0 kb and 4.7 kb respectively³³ and by reference to tobacco mosaic virus (6.34 kb)³⁴ and brome mosaic virus (0.87, 2.3, 3.1, 3.4 kb)35 RNAs fractionated in adjacent lanes.

Different portions of these 'very distant' 3' sequences may be used in the 3'-untranslated regions of these δ_m mRNAs analogous to the variable 3'-untranslated regions of the multiple forms of mRNA for mouse dihydrofolate reductase²³. Alternatively, one or more exons in the sequence may be translated and expressed as alternative or additional 3' polypeptide termini. Such different forms of δ_m chain might thus allow not only a transmembrane 'anchor' for membrane IgD but also one or more internal (cytoplasmic) termini available for different effector functions of IgD. Another possibility is that the multiplicity of δ_m mRNAs represents a redundancy in the δ gene to

ensure that B cells will be able to produce membrane IgD even if genetic accidents interfere with mRNA processing and/or translational pathways.

Note, however, that at least some of the δ_m mRNAs in the plasmacytomas are different from those identified in normal spleen. AC1 and DC1 sequences are readily detectable in the plasmacytoma RNAs but not in normal spleen. The lack of AC1 and DC1 hybridization in spleen RNA probably reflects the fact that spleen primarily synthesizes δ_m and contains very few plasma cells which utilize RNA transcriptional pathways leading to δ_s expression. It has been postulated^{2,3} that deletion

Fig. 3 Restriction enzyme map showing the BALB/c δ gene¹⁶ and the genomic fragments subcloned from it. B, BamHI; C, ClaI; H, HindIII; M, MboI (converted to BamHI when inserted into Charon 28 phage clone); N, HincII; P PvuII; R, EcoRI; S, RsaI; and X, Xbal. The restriction enzyme map and the boxes defining δ exons are described in greater detail elsewhere 16,17. Fragments from phage clones of liver DNA 16 indicated diagrammatically here were subcloned into appropriate plasmid vectors. The preparation of the $C\delta$ probe is described in Fig. 1 legend. Before nick-translation²⁶ the re-



combinant DNA fragments were separated from the plasmid vector by restriction endonuclease digestion and fractionation on polyacrylamide . A semiquantitative diagram summarizing the results of repeated experiments in which plasmacytoma RNA or spleen RNA was hybridized with each probe is presented below the name of each probe. Photographs of representative autoradiograms are presented in Fig. 2, but many of the minor bands are difficult to photograph in the presence of other bands which hybridize very strongly. VDC3 gave the same hybridization pattern as VDC2, and AC2 and AC3 did not reveal any discrete bands of hybridization (data not shown). Two fragments were prepared from VDC1 that contain the following sequences: those between HincII and RsaI, or those between RsaI and ClaI. Both fragments gave the same pattern of hybridization as the whole VDC1 (data not shown).

of the μ gene is necessary for cells to synthesize secreted IgD. The rearrangement 5' to $C\delta 1$ in TEPC 1017 and TEPC 1033 is consistent with such a deletion, and it could also alter transcriptional and processing pathways in the δ_m gene complex.

Membrane IgD has long been thought to be important in B-cell development although its exact role remains controversial. As discussed above, the multiple δ_m mRNAs described in this report could represent mRNAs for multiple forms of δ_m protein. Cloning of DNA sequences for these multiple $\delta_{\rm m}$ mRNAs and cell-free translation studies are in progress to help elucidate the biological role of membrane IgD and the regulation of its expression.

We thank Fred D. Finkelman and our colleagues in the NIAID Laboratory of Immunology and the NCI Laboratory of Cell Biology for critical reading of the manuscript. We thank Shirley Starnes for assistance in preparing the manuscript. The work was supported in part by NÎH grants AI 16547 to P.W.T. and GM 21812 to F.R.B.

Received 14 December 1981, accepted 5 February 1982

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Xenobiotic conjugation: a novel role for bile acids

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The conjugation of foreign compounds with hydrophilic natural products (for example, amino acids, glucuronic acid) represents an important biochemical mechanism of attenuating xenobiotic toxicity and of facilitating excretion of hydrophobic chemicals. Although several conjugation reactions are known¹, bile acids have not been implicated previously in the conjugation of xenobiotics. The physiological importance of natural bile acids is based partly on their propensity to conjugate through their carboxyl groups with several amino acids (especially glycine and taurine), thereby creating effective emulsifying agents to aid the digestion of lipids. However, we now report that in several animal species, bile acids form metabolic conjugates through a hydroxyl group with an acidic metabolite of fluvalinate, a pyrethroid insecticide. Conjugates of this acidic metabolite with cholic, taurochenodeoxycholic and taurocholic acids are major secondary metabolites found in excreta of the cow, chicken and rat, respectively, and represent 5-12% of the faecal 'C-labelled residue.

Fluvalinate (\alpha-cyano-3-phenoxybenzyl 2-(2-chloro-4-trifluoromethylanilino)-3-methylbutanoate; Mavrik) is a pyrethroid insecticide2 that is metabolized primarily by ester hydrolysis to an anilino acid (Table 1 and Fig. 1), and is excreted intact, conjugated with amino acids, or hydroxylated at the isopropyl methyl group^{3,4}. Using TLC and reverse-phase liquid chromatography, we isolated from cow faeces an unknown metabolite of fluvalinate which was identified by mass spectrometry as an anilino acid conjugate of cholic acid (Table 1).

Table 1 Bile acid conjugates of an acidic xenobiotic in animal faeces

				% ¹⁴ C in faeces		
Conjugate	R_1	R ₂	R_3	Rat (0-3 day)	Chicken (0-1 day)	Cow (3–4 day)
Cholate	ОН	OH	O	0.5	< 0.5	8.1*
Chenodeoxycholate	он	H	O		*****	1.3
Deoxycholate	H	ОН	O ⁻			2.2
Taurocholate	OH	ОН	NHCH ₂ CH ₂ SO ₃	5.0	< 0.5	< 0.5
Taurochenodeoxycholate	OH	H	NHCH ₂ CH ₂ SO ₃	3.2	11.9†	< 0.5
Taurodeoxycholate	H	OH	NHCH ₂ CH ₂ SO ₃	< 0.5	< 0.5	< 0.5
Glycocholate	OH	OH	NHCH ₂ CO ₂	< 0.5	< 0.5	0.3
Glycochenodeoxycholate	OH	H	NHCH ₂ CO ₂	< 0.5	< 0.5	0.6
Glycodeoxycholate	H	ОН	NHCH ₂ CO ₂	<0.5	< 0.5	0.2

* The choic acid conjugate when methylated (diazomethane in ether/methanol) displayed the following characteristics. TLC (silica gel GF), $R_F = 0.28$ m ether/hexane (1·1), reverse-phase liquid chromatography (LiChrosorb RP-8 column, acetonitrile/0.1% acetic acid (75·25), flow rate 1.6 ml min⁻¹, UV detection at 254 nm, k' = 12.7); mass spectrum (m/z (relative intensity), electron impact), 701 (1.1, M⁺ for Cl = 37), 699 (3, M⁺ for Cl = 35), 252 (97), 250 (100), mass spectrum (chemical ionization (CH₄)), 728 (5, M+29), 700 (6, M+H for Cl = 35).

The taurochenodeoxycholic acid conjugate (as its methyl ester) was purified by TLC (silica gel GF, ethyl acetate/hexane (2.1)) and reverse-phase liquid chromatography (methanol/0 1% acetic acid (80 20), k'=15 5). It was identified from the following spectral data ¹H NMR (Bruker WM-300, 300 MHz, (CDCl₃)), $\delta=0.66$ (s (singlet), 3, CH₃ at C-18), 0.91 (s, 3, CH₃ at C-19), 1 03 (d (doublet), 3, J=7 Hz, isopropyl CH₃), 1.08 (d, 3, J=7 Hz, isopropyl CH₃); 3.74 (t, 2, J=6 Hz, CH₂SO₃), 3.93 (s, 3, OCH₃), 4 66 (multiplet, 1, H at C-3 of steroid). Field desorption mass spectrum (m/z (relative synthesized for analysis by ¹H NMR spectroscopy (Varian T-60 instrument, CDCl₃) to determine the position of conjugation on the bile acid For each standard the chemical shift of the methine hydrogen in the bile acid at the carbon bearing an acylated hydroxyl was used to diagnose the site of esterification: for the anillino acid conjugate of methyl inthocholate (3β -methine hydrogen only), $\delta=4.80$ p.p.m (broad singlet); for the trisbenzoate of methyl cholate, $\delta=4.82$, 5 38, 5 50 (3β -, 7β - and 12 β -methine hydrogens, respectively); for the bisbenzoate of methyl deoxycholate, $\delta=4.90$ and 5.43 (3β - and 12 β -methine hydrogens, respectively). Together with the broadness of the 3β -methine hydrogen resonance (compared with that of the 7β - and 12 β -hydrogens), these data support our assignment of conjugation at the 3α -hydroxyl monety.

Subsequent comparison with a synthetic standard confirmed this.

The apparent novelty of this bile acid adduct led us to examine the generality of the finding. Most (67%) of the ¹⁴C-labelled metabolite residue in excreta of chickens treated with [CF3-¹⁴Clfluvalinate was highly polar (no migration on silica gel TLC with development in ethyl acetate). However, methylation of this polar 14C-residue with diazomethane gave (in part) a relatively nonpolar product ($R_F = 0.25$ on silica gel GFTLC developed with ethyl acetate/hexane, 2:1). By administering a single oral dose of ¹⁴C-fluvalinate (100 mg per kg) to a Leghorn hen, we purified 300 µg of this metabolite (as its methyl ester) using TLC and reverse-phase liquid chromatography. The metabolite was identified as an anilino acid conjugate of taurochenodeoxycholic acid by ¹H NMR spectroscopy and field desorption mass spectrometry. Acylation of taurochenodeoxycholic acid by the anilino acid occurred at the 3α -hydroxyl group (not the 7α -hydroxyl), as deduced from the ¹H NMR chemical shift of the methine hydrogen at C-3 of the acylated bile acid ($\delta = 4.66 \text{ p.p.m.}$) and its multiplet coupling pattern. A synthetic sample of the conjugate gave an identical NMR spectrum, showing that chemical and metabolic acylation both favour the least hindered 3α -hydroxyl position of the bile acid5.

To determine any additional bile acid conjugates, the remaining common bile acids (as methyl esters) were acylated using

the acyl chloride of the anilino acid, thus providing reference standards for chromatographic comparison. Rats, chickens and a cow were given a single oral dose of fluvalinate at 1 mg per kg; analysis of their excreta by TLC, and reverse-phase and normal-phase liquid chromatography, revealed a general occurrence of these bile acid conjugates (Table 1). The relative abundance of a particular conjugate appeared to be proportional to the composition of the natural bile acids in the animal: for example, taurochenodeoxycholic acid is the major bile acid in chickens while taurocholic and taurochenodeoxycholic acids are most abundant in rats⁶.

The fact that bile acid conjugates of xenobiotics have not been described previously may be explained by the numerous difficulties in manipulating these conjugates. In this study the taurine-containing conjugates were most abundant—these compounds are highly polar because of the free sulphonic acid moiety, adhering tightly to silica gel and even laboratory glassware. Methylation of the sulphonic acid is inefficient and once formed, the methyl ester decomposes slowly whether neat or in acetone solution.

We have described a new, apparently general role for bile acids in the metabolic conjugation of a xenobiotic by a rodent, a ruminant and a bird. Although this example involves a pesticide metabolite, certain drugs (or their acidic metabolites) may be processed similarly. Indeed, normal resorption of these bile acid conjugates may offer an alternative

Fig. 1 Conjugation of fluvalinate to an anilino acid.

explanation for some enterohepatic circulation of foreign com-

We emphasize that these bile acid conjugates are not minor metabolites; collectively they represent 6 and 11% of the administered 14C dose in rats and chickens, respectively. Our suggestion that they have general occurrence is based largely on the promiscuous conjugation of an anilino acid with whatever bile acids predominate in the species investigated. Further studies should test the generality and toxicological ramifications of this excretion mechanism and may also identify previously unknown, intractable metabolic residues.

We thank G. C. Jamieson for mass spectral analysis; Drs M. Maddox and C. Schramm for ¹H NMR analysis; and Drs Y. Naya and H. Naoki for field desorption mass spectrometry.

Received 11 January, accepted 5 February 1982

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Evidence for the lipidic nature of tight junction strands

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We examine here the proposition that membrane lipids¹⁻⁴, rather than intrinsic membrane proteins⁵⁻⁷, are the principal structural elements of the strands comprising tight junctions. Our evidence, which is based on direct rapid freezing of newly formed tight junctions between rat prostate epithelial cells, indicates that individual tight junction strands are pairs of inverted cylindrical micelles sandwiched between linear fusions of the external membrane leaflets of adjacent cells. Although individual tight junction strands appear as continuous cylinders when fractured near the frozen surface, where ice crystals have not damaged the plasma membrane, they appear as rows of particles when fractured deeper in the frozen tissue. We now interpret these tight junction particles as remnants of intramembrane cylinders disrupted during freezing The morphology and dimensions of the intact cylinders correspond to those of lipids in the cylindrical hexagonal II phase^{8,9} and this suggests that tight junction formation requires a phase transition of the planar lipid bilayer similar to that invoked in models of membrane fusion^{10,11}. Our morphological interpretation explains the known functional properties of tight junctions.

Tight junction strands have been assumed to consist of a row of intramembrane particles representing integral membrane proteins which on fixation (glutaraldehyde 1-5%) and cryoprotection (glycerol 20-30%) form the continuous fibrils observed in standard freeze-fracture replicas^{5-7,12-16}. This interpretation was based on the observation that a chain of particles replaces the continuous fibrils when the only preparative step is freezing by immersion in liquid Freon—a procedure which freezes tissue more slowly than current rapid freezing methods 12,17. Subsequent studies where mixtures of fibrils and particles were obtained after brief fixation in dilute glutaraldehyde were interpreted to confirm the proteinaceous nature of tight junction strands7. Freeze-fracture has also been used to investigate whether the tight junction strand comprises a single fibril¹⁸ or a pair of fibrils, either lying side by side^{5,14} or, as most recently suggested, offset across the membrane contact⁶.

In the present experiments, fresh tissue was frozen directly with liquid helium in order to minimize the physical effects of ice crystal formation and to avoid the effects of chemical fixation and cryoprotection with glycerol (which may contain contaminants with potential cross-linking properties⁷). Pieces of ventral prostate gland were excised from adult Sprague-Dawley rats under light ether anaesthesia. The slices were incubated for 3-15 min in 0.1 M buffer, pH 7.4, at 37 °C, placed on a rapid freezing stage and frozen with liquid helium by methods described elsewhere¹⁷. During this incubation, new tight junction strands form along the entire length of the lateral plasma membranes of adjacent epithelial cells2.

Near the original surface of the slice, where ice crystals do not impinge on the plasma membrane¹⁷, pairs of cylinders (9-11 nm in diameter) lie side by side along contacts between adjacent bilayers; each cylinder corresponds to a tight junction fibril. The cylinders form elongated segments that adhere to either the protoplasmic (P, Fig. 1a) or the exoplasmic (E, Fig. 1b) fracture face, leaving a complementary furrow on the other fracture face (Fig. 1a,c,d). Where the plane of fracture jumps from an E face of one membrane to the P face of the adjacent membrane two cylinders may be exposed (Fig. 1d) or one cylinder and a groove left by an adjacent cylinder that adhered to the complementary E face (Fig. 1c). For such planes of fracture to occur, the cylinders must be slightly offset with respect to each other⁶. This asymmetric disposition of the cylinders also explains why lateral views at junctional P face-P face transitions between abutting membranes of adjacent cells show only one of the cylinders, regardless of whether the transition is viewed from its apical or basal aspect (Fig. 1e). The diagram of a tight junction strand in Fig. 2a illustrates in cross-section the disposition of the two intramembrane cylinders. Figure 2b shows different observed planes of fracture through such a

Deeper in the frozen tissue, where larger ice crystals impinge on the plasma membrane, cylinders are replaced by chains of particles that always appear on the E faces (Fig. 1f), but still leave continuous grooves on the P faces (Fig. 1g). Short segments of cylinder mixed with rows of particles are found over continuous grooves in both E (Fig. 1h) and P (Fig. 1i) faces of the plasma membrane at intermediate levels of freezing. The chains of particles seen on the E fracture faces of plasma membranes in poorly frozen areas (Fig. 1f) appear to be remnants of disrupted cylinders. Additional evidence that the tight junction strand is not a chain of particles is that a furrow, not a row of punctate depressions, is found on the complementary P face (Fig. 1g).

Although freeze-fracture images are normally analysed on the assumption that particles represent intramembrane proteins, it has recently been demonstrated that membranes made of lipids, which are capable of forming hexagonal II phase, have pits and particles on their freeze-fracture faces 1,11,19-23. Indeed, some published micrographs of protein-free liposomes suggest the presence of elongated cylinders within the lipid bilayers (see Fig. 1b in refs 23 and 24). The intramembrane cylinders at tight junctions have several features, such as hydrophobic environment (interior of the bilayer), morphology (smooth surface) and dimensions (9-11 nm diameter), which resemble those of inverted cylindrical lipid micelles (hexagonal II phase)^{8-10,25-27}, and which suggest that the cylinders found after direct rapid freezing could be differentiated lipidic domains rather than proteinaceous structures embedded in the mem-

Other evidence that tight junction strands are lipidic, rather than chains of protein particles, is (1) cylinders obtained after glutaraldehyde fixation of pancreas or prostate tissues are disrupted after soaking in glycerol4 or acetone (B.K. and T.S.R., unpublished observations) but neither glycerol or acetone

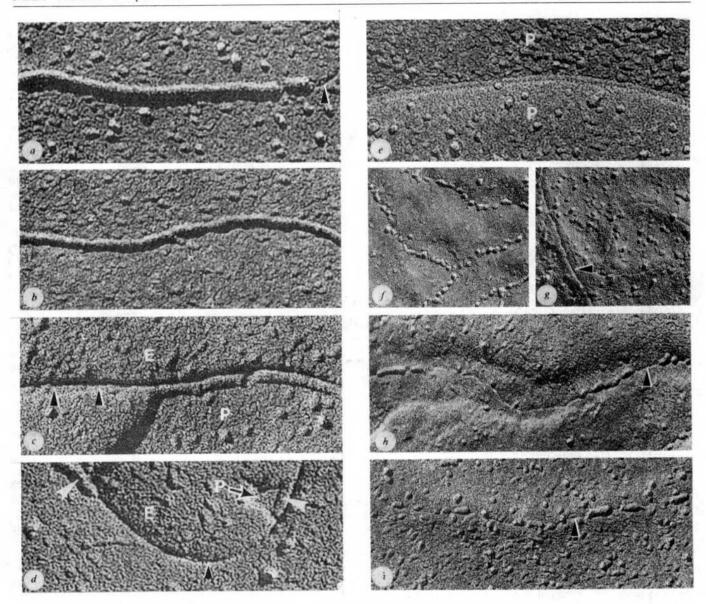


Fig. 1 Tight junction strands on fracture faces of plasma membranes of directly frozen rat ventral prostate epithelial cells. Continuous cylinders are found on P (a, c) and E (b) fracture faces near the original surface of the tissue where ice crystals have not visibly damaged the membranes. Two cylinders, one belonging to each of the pair of membranes participating in the tight junction, are seen in d (white arrowheads). Cylinders are also typically intact at junctional P face-P face transitions at abutting basal portions of plasma membranes of adjacent cells (e). At deeper regions in the specimen, where ice crystals impinge on membrane structure, chains of particles replace cylinders on E fracture faces (f), leaving a continuous groove on P faces (g). Mixtures of particles and short segments of cylinders are found over continuous grooves in both E (h) and P (i) faces from regions of intermediate ice crystal damage. P, P fracture faces; E, E fracture faces; (g) point to continuous grooves. Platinum deposits are white. (g) and (g) and (g) and (g) and (g) are (g) and (g) are (g) and (g) and (g) and (g) are (g) are (g) are (g) are (g) and (g) are (

would be expected to break covalent bonds formed by reaction of glutaraldehyde with integral membrane proteins. It is not even clear why glutaraldehyde fixation would affect the structure of the tight junction strand at all, unless it cross-links cytoplasmic28 or peripheral membrane proteins associated with the tight junction strands. (2) Tight junction strands form within minutes in the absence of protein synthesis and without any noticeable change in the density or pattern of distribution of the intramembrane particles2. However, punctate dimples and very short segments of tight junction strands are occasionally observed in plasma membranes of specimens rapidly frozen just as tight junction proliferation begins. We tentatively regard these dimples as early steps in tight junction formation because similar punctate dimples represent points of contact that become the sites of monolayer and bilayer rupture required for membrane fusion between artificial protein-free bilayers^{23,27}.

Transitions from planar bilayer to cylindrical micelles are also invoked in models of bilayer fusion 10,11,21,27. In these transi-

tions, the area of the polar side of the monolayer is thought to decrease while that of the hydrocarbon chain layer expands, so that the lipids pack into highly curved inverted cylindrical micelles with outward-facing hydrocarbon chains. A similar process could lead to tight junction formation; segregation and packing of lipids during the phase transition would account for the formation of the intramembrane cylinder. These changes might involve a transition of the planar lipid bilayer to a cylindrical micelle in an hexagonal II phase, similar to that invoked in models of membrane fusion, but here somehow stabilized in the form of pairs of offset, inverted cylindrical micelles segregated within an interbilayer fusion. Figure 2c illustrates in cross-section the proposed micellar organization of the lipids at the tight junction strand. Figure 2d shows how this model can be applied to the interpretation of a freeze-fracture image.

The presence of a continuous cylinder intercalated between two protoplasmic faces as seen in apical or basal views of tight junctions (Fig. 1e) requires that there be a continuous, linear

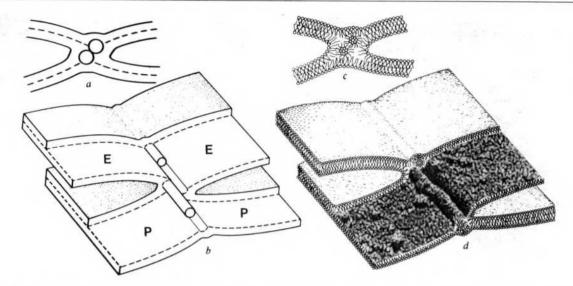


Fig. 2 a, Diagram of a cross-section of a tight junction strand illustrating the offset disposition of the pair of intramembrane cylinders. b, Diagram illustrating the paired offset cylinders at a tight junction strand and the different possible planes of fracture through such a junction. c, Proposed organization of the phospholipids at a tight junction strand. d, Diagram of phospholipids combined with freeze-fracture micrograph to show how fractures through lipid micelles could produce images characteristic of tight junctions,

interruption of the exoplasmic halves of the bilayer around an individual cell (Fig. 2b). However, the presence of inverted cylindrical micelles inside the membrane would also require continuity of the exoplasmic halves of bilayers of neighbouring cells across the tight junction strand (Fig. 2c). In fact, thin sections across tight junctions show points of partial membrane fusion, where only the exoplasmic leaflets of adjacent membranes have become continuous^{5,29,30}. Also, fluorescence quenching experiments show that lipid probes limited to the exoplasmic leaflets of cells joined by tight junctions are prevented from passing from their apical to their basal surface, while lipid probes that reach the protoplasmic leaflet can move freely around the cell circumference31. Thus the interpretation of tight junction structure presented here is consistent with their role as barriers that prevent diffusion between extracellular spaces and isolate apical and basal regions of the plasma membrane^{5,31}. It also shows how lipids and possibly other membrane components might flow from cell to cell within shared exoplasmic membrane leaflets.

Bryan Schroeder performed the photographic work, Nicholas Christakis helped with laboratory work, Trudy Nicholson prepared Fig. 2, and Sandra Cotter typed and provided editorial assistance. We especially thank Peter Rand and Richard Ornberg for valuable discussions during preparation of the manuscript.

Received 26 October 1981; accepted 11 February 1982.

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The structure of a pseudo intercalated complex between actinomycin and the DNA binding sequence d(GpC)

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Actinomycin D (AMD) is used clinically to treat tumours such as Wilms' tumour and gestational choriocarcinoma. It inhibits transcription in most cellular systems3,4, and binds to DNA, not to RNA3-5, with a preference for guanine6. The study of the crystal structure of a 2:1 complex between deoxyguanosine and AMD demonstrated both stacking and hydrogen-bonding interactions between the drug and the guanine ring7. Solution studies8,9 have indicated that the drug binds preferentially to guanine-pyrimidine sequences, such as d(GpC), and that an intercalated complex forms with both DNA¹⁰ and DNA fragments¹¹. External binding¹² and intercalation^{10,13} models for the structure of the complex between AMD and DNA have been proposed, but until now no crystal strucutre of a complex between AMD and an oligonucleotide has been reported. As the smallest unit of DNA with the potential for forming an intercalated duplex is a self-complementary deoxydinucleoside monophosphate, we undertook the crystallographic analysis of the 2:1 complex between deoxyguanylyl-3', 5'-deoxycytidine, d(GpC), and AMD. The complex is found to form an unusual pseudo-intercalated structure.

Crystals of the complex were produced from aqueous solution with 3 mM d(GpC) and 1 mM AMD, using a vapour diffusion method. The red-orange crystals, some of which were thin hexagonal plates and others tetragonal rods, appeared after about 1 month. Spectroscopic analysis of dissolved crystals revealed a 2:1 ratio of d(GpC) to AMD. X-ray diffraction studies showed the hexagonal crystals to be isomorphous with the tetragonal ones and indicated a trigonal crystal system with cell dimensions a = b = 16.577(3) Å and c = 56.200(8) Å. The intensities were measured at 0 °C on a diffractometer using 75uK α radiation and a co-axial gas flow cooling system. Reflection intensities were measured in one octant $(h\bar{k}l)$ of reciprocal space with $2\theta \le 62^{\circ}$ (1.5 Å resolution), using a $\theta/2\theta$ scan technique. A total of 3,706 reflections were collected and their intensities were corrected for absorption¹⁴ and decay. The data were then averaged assuming point group 3m symmetry yielding 1,668 independent reflections. A systematic absence is observed in the 00l zone with $l = 3n \pm 1$, thus the four possible space groups are P3₁21, P3₁12, P3₂21 and P3₂12. For the 1.5 Å resolution data the percentage of observed reflections on $1\sigma(\text{Fo}^2)$ and $2\sigma(\text{Fo}^2)$ levels are 74% and 58% respectively. From the observed crystal density (1.36 g cm⁻³) and unit cell volume (13,374.5 Å³), three complexes (molecular weight 2,366) and about 215 water molecules were predicted to be in the unit cell. All possible space groups require that the complex use the crystallographic 2-fold axis. AMD itself does not possesss exact 2-fold symmetry, hence its amino and quinoid groups are statistically disordered.

The structure was determined by inspection of the Patterson map and computerized electron density fitting procedures¹⁵. Refinement procedures resolved the space group ambiguities. After several cycles of constrained-restrained least-squares refinement, water molecules began to appear in the difference Fourier maps and were added to the structure; most of them are disordered. The current R-factor is 19% for all data and 15% for 2σ data. The fractional coordinates of the complex have been deposited in the Cambridge data file.

The principal and surprising feature of the crystal structure (Figs 1a and 2a, b) is that it does not contain the intercalated duplex structure (Fig. 1b) proposed, for example, by Sobell and Jain¹³ on the basis of the crystal structure of the 2:1 complex between deoxyguanosine and AMD⁷. Instead the d(GpC) molecule is in the opened-out conformation with the phosphodiester torsion angles in the g⁺g⁺ range rather than the g⁻g⁻ range found in self-complementary duplexes 16-21; thus its conformation is similar to that displayed by ApU²² complexed with 9-amino acridine. The dinucleoside phosphate molecules are connected to each other via Watson-Crick base pairs, to form infinite chains, with each hydrogen-bonded d(GpC) chain making a right-handed spiral around the crystallographic screw axis. Each AMD molecule, whose structure is very similar to the one complexed with deoxyguanosine alone⁷, is located between these chains with its phenoxazone ring sandwiched between Watson-Crick base pairs from these different chains. The complex is thus pseudo-intercalated. In the AMD binding site, the phenoxazone ring stacks predominantly with both guanine rings and has very little overlap with the cytosines (Fig. 3). The guanine residues also interact with the cyclic peptide rings of AMD via hydrogen bonds between their amino group N-2 and ring nitrogen N-3 and the carbonyl oxygen and amide group of threonine residues respectively (Fig. 2). The same intercomplex hydrogen bonds were found in the 2:1 complex between deoxyguanosine and AMD⁷

It is not yet clear why the d(GpC) does not form a duplex in the crystal because there is no chemical feature, such as base protonation, that would preclude such a structure. However, the rigid requirements for drug binding are incompatible with the conventional intercalated structures²¹, because the base pairs must form an unusual stacking pattern with the phenoxazone ring (Fig. 3) for the guanine to be positioned correctly for hydrogen bonding with the threonine (Fig. 2). Although we were able to build a stereochemically acceptable

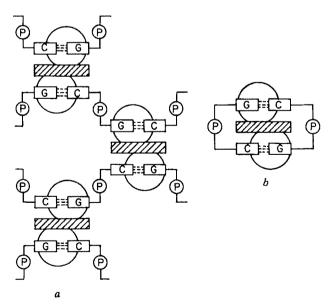
intercalated model, the conformation of the dinucleoside phosphate backbone is unusual. This may explain in part the unusually large ³¹P-NMR downfield shifts observed in complexes between AMD and small oligomers^{23–25}. These results also support the view that intercalation alone has a sequence preference at the dimer level. Although the crystal structures of both GpC¹⁶ and ApU¹⁷ show double-helical conformations, when ApU is complexed with 9-amino-acridine²² and when the deoxy form of GpC is complexed with AMD, duplexes do not form in the crystal. To date, without exception, all the known crystal structures of duplex-intercalated drug complexes have pyrimidine-3', 5'-purine sequences²¹ including the daunomycin hexamer complex²⁶. Furthermore, potential energy calculations for dinucleoside phosphates²⁷ indicate that standard intercalation geometries appear to favour that sequence.

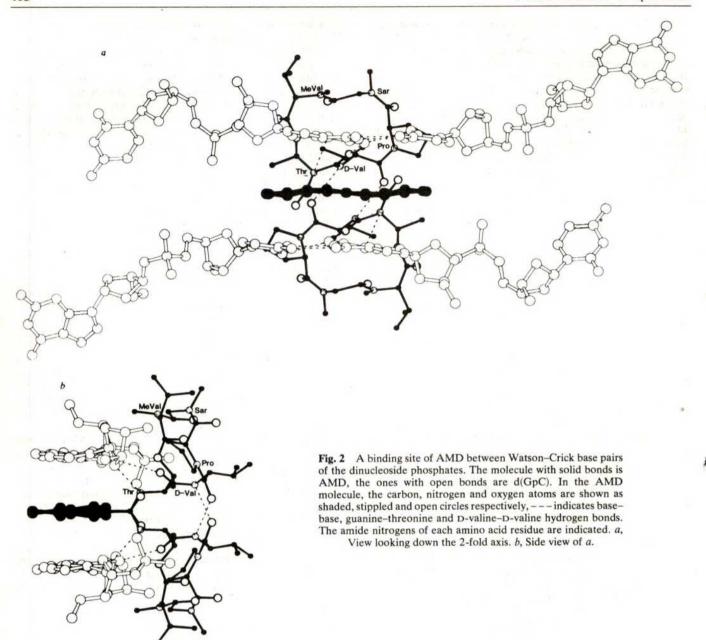
tion geometries appear to favour that sequence.

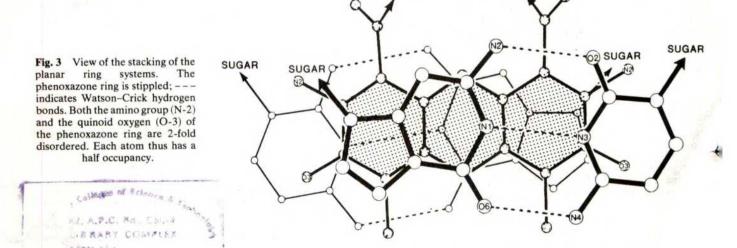
This analysis supports the proposals 10,12,13 that two 2-amino purines, such as guanine, are important, if not always essential²⁸ for the binding of AMD to DNA. The AMD molecule hydrogen-bonds only to guanine and not to cytosine, and it is the guanine base that is predominantly stacked with the phenoxazone ring (Fig. 3). Furthermore, only one of four possible orientations of the Watson-Crick G · C base pair allows hydrogen bonds to form between the cyclic peptide and the amino group N-2 and ring ntirogen N3 of the guanine ring. From considerations of base-drug interactions alone it would be predicted that AMD could bind to duplex d(GpC) but not to duplex d(CpG) because only the former sequence meets the hydrogen-bonding requirements. On the other hand, d(CpG) in an open conformation can complex with AMD⁸. In this structure the cytosine serves only as an accessory in anchoring the guanine in an optimal position for binding to AMD.

An examination of this structure shows why AMD cannot bind to RNA. If the deoxyribose of the guanosine (and not the cytidine) were changed to a ribose sugar, the O-2' hydroxyl group would have unusually short contacts with the amino group N-2 of the phenoxazone ring. This would also be the case in an intercalated model¹³ in which the distance between C-2' and the amino nitrogen N-2 is already quite short; it would thus be clearly impossible to replace the guanosine deoxyribose by ribose.

Using the results of this analysis it is possible to build a model of DNA in which guanine and cytosine residues from adjacent helices are involved in hydrogen-bonded cross-linking that is







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stabilized by AMD. Further studies are necessary to determine whether this type of structure can exist in oligo- or polynucleotide-AMD complexes and if such structures are biologically significant. In recent studies²⁹ of semisynthetic AMD derivatives where both amino N-2 and C-7 sites of the phenoxazone ring were modified by substituting bulky groups for hydrogen atoms, the analogues were found to be effective antitumour agents, to inhibit DNA and RNA synthesis and to bind to DNA. In these cases it is difficult to envision a sterically feasible intercalation model that also incorporates the peptide-base hydrogen bonds; however, it is easy to fit these semisynthetic AMDs into a cross-linked structure, suggesting an alternative explanation for a part of AMD's chemotherapeutic action. Cross-linking may also provide an explanation for some of the unusual ³¹P-NMR results observed with short DNA oligomers in solution²³⁻²⁵. Clearly not all the activities of AMD need be explained by classical intercalation.

This research was supported by grants from NIH (GM-21589, CA-22780, CA-06927, RR-05539) and the Cancer Research Campaign (SP-1384), and by an appropriation from the Commonwealth of Pennsylvania. S.N. is a Career Development Awardee of the Cancer Research Campaign. We thank Miss Kristie Kunkel for her assistance, and Drs Thomas Krugh, Dinshaw Patel and Peter Young for discussions.

Received 19 October 1981, accepted 9 February 1982

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A gene deletion ending at the midpoint of a repetitive DNA sequence in one form of hereditary persistence of fetal haemoglobin

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The form of hereditary persistence of fetal haemoglobin (HPFH) that commonly occurs in black populations is an inherited disorder of haemoglobin synthesis characterized by a uniformly high level of fetal haemoglobin (HbF) synthesis in all the erythroid cells of affected adult individuals^{1,2}. The precise molecular basis of the HPFH phenotype remains unknown, but is of great interest because of the knowledge that could be gained, through its understanding, of the mechanisms that regulate the expression of globin genes during development. The most common form of HPFH in black populations is associated with an extensive deletion that includes the normal adult (δ and β) globin genes and adjacent flanking DNA³⁻⁸. To investigate this disorder in more detail, we have cloned the DNA encompassing the region of the gene deletion in a case of HPFH and have determined the nucleotide sequence across the 5' end point of the deletion within the non- α -globin gene complex. We report here that this end point maps at the midpoint of a member of the 'AluI' family of repetitive sequences located approximately 4 kilobases (kb) to the 5' side of the δ -globin gene. Such repetitive sequences may be 'hot spots' of recombination, and are possibly involved in regulating gene expression.

The isolation and characterization of the HPFH DNA clone will be described elsewhere (D.T., S.M.W. and B.G.F., in preparation). The restriction endonuclease map of the human

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non- α -globin gene cluster in the region of the HPFH deletion end point that was cloned is shown in Fig. 1. The deletion end point occurs \sim 4 kb 5' of the δ -globin gene. The normal DNA flanking the 5' extremity of δ -globin gene that was cloned by Lawn et al.9 was subjected to nucleotide sequence analysis in our laboratory and was shown to contain the following noteworthy structural features (see also Fig. 1); (1) an inverted pair of 300 nucleotide-long repetitive DNA sequences of the 'AluI' family (refs 10-14) separated by ~700 bp of intergenic DNA and located \sim 4 kb from the 5' end of the δ -globin gene, and (2) a remarkable segment of DNA located ~1 kb from the 5' end of the δ -globin gene that contains (with one exception) over 200 successive pyrimidine nucleotides on one strand of the DNA12

Figure 2 compares the nucleotide sequences of normal and HPFH DNA starting at the 5' EcoRI site of the 3.1-kb EcoRI DNA fragment adjacent and 5' to the 2.25-kb EcoRI DNA fragment bearing the 5' end of the δ -globin gene (Fig. 1; refs 9, 13). The two sequences show perfect homology with only two base differences as far as residue 370 from the 5' EcoRI site (Figure 2); beyond this point there is only very limited, patchy homology between the two DNA sequences, Figure 2 shows that the normal sequence retained in the HPFH patient extends half-way through the upstream (5') member of the pair of inverted Alu family DNA sequences located 5' to the δ globin gene. Thus the deletion in HPFH removes >3,000 nucleotides of the DNA flanking the 5' end of the δ -globin gene, including one and a half members of the pair of inverted Alu family repetitive DNA sequences and an extensive polypyrimidine stretch, then extends through the δ - and β -globin genes for an unknown distance 3' of the β -globin gene. The 3' Alu family DNA sequence that is totally deleted in this case of HPFH is known to be transcribed in vitro by RNA polymerase III to yield an RNA molecule 490 nucleotides long (although it is unknown whether such transcription occurs in vivo), whereas the 5' Alu sequence that is half deleted is not transcribed in vitro 10,11,14; the transcriptional orientation of this 3' Alu sequence is the same as that of the δ -globin gene.

It has been postulated 15 that the inter-γ-δ gene DNA may contain control elements, the deletion of which is responsible for the HPFH phenotype. Potential candidates for such regula-

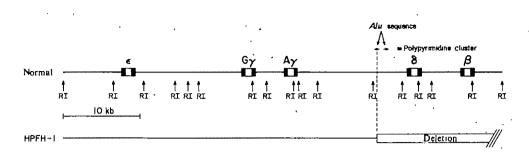


Fig. 1 Schematic representation of human non-α-globin gene cluster, showing the location of the deletion involved in the studied case of hereditary persistence of fetal haemoglobin (HPFH-1) Vertical arrows indicate EcoRI restriction endonuclease cleavage sites. Solid boxes show coding regions, and open boxes the large intervening sequences of the globin genes. 'Alu sequence' indicates the two copies of moderately repetitive DNA sequences of the AluI family located 5' to the δ globin gene.

tory sequences are the Alu repetitive DNA sequences and/or the polypyrimidine tract described above, although the specific molecular mechanisms by which deletion of these DNA elements could mediate failure of postnatal repression of the fetal γ -globin genes only on the cis chromosome bearing the deletion, are unknown and remain the subject of speculation. On the other hand, the coincidence of the deletion end point with an Alu family repetitive DNA sequence may indicate that Alu sequences, because of their number, chromosomal distribution and sequence homology, can serve as 'hot spots' for intrachromosomal recombinational events that lead to DNA deletions. Such recombinational events would be expected to occur between two homologous Alu sequences and result in a fusion product containing portions of the two Alu sequences involved in the nonhomologous cross-over. In HPFH, however, only half of an Alu sequence persists as opposed to a full-length fused Alu sequence. One must therefore implicate other mechanisms for the loss of the other half of the expected fused Alu sequence. It is also possible that a cross-over occurred between two relatively nonhomologous DNA sequences on the

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TIAGTAGATA GÖGGGTTTCA CCATGITGGT CAGGCTGATC TCGAACTCCT AACATCAGGT
       GATCCACCCI LCTCBGCCIL IBAAAGTGLT GGGATCACAG GCGGTGAGCC ACCACACCCA
       SCTOCTOCIC TIGECCCIA SCAGITCCIT CTCTCIAGAT STICCSTOSS STOTSTIGGS
      GCCAAGAATG TGAATTITGE Normal
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Fig. 2 Comparison of normal inter- γ - δ gene DNA sequence (upper line) with that of the DNA across the 5' end point of the deletion in HPFH (lower line). Asterisks show positions at which an identical nucleotide is found in both sequences. The short arrows overlying the sequence indicate the direct repeats that flank the extremities of the normal AluI DNA sequence and the solid line overlies the entire length of the normal AluI repetitive DNA sequence.

chromosome bearing the non α -globin genes. Only limited spotty homology has been previously noted for DNA sequences involved in various recombination events between simian virus 40 (SV40) viral DNA and host genomic DNA¹⁶

Two other cases involving presumed recombination within AluI repetitive DNA sequences are known; one where a portion of an AluI repetitive DNA sequence from the genomic DNA of infected monkey kidney cells has been incorporated into SV40 (ref. 17) and another in which an α -globin gene has been partially deleted and fused to a repetitive DNA sequence of the AluI family¹⁸. In both cases, the recombinational events occurred internally within the AluI repetitive DNA sequences rather than at their boundary. It is not clear whether such occurrences are coincidental because of the high frequency of the AluI family sequences in the total genome, or whether there is some special propensity for nonhomologous recombination to occur within DNA stretches containing AluI repetitive sequences. Note, in this regard, that a number of deletions have been found in a yeast tRNA gene cluster that is flanked by short repetitive DNA sequences 19

Finally, because there is another deletion producing HPFH in blacks that has a different 5' end point extending across the same region of DNA^{7,8}, it seems unlikely that the HPFH phenotype in these cases is due to a second undetected mutation remote from the region of the deletion. It is possible, however, that the size of the deletion itself, the loss of downstream sequences, or the nature of the DNA brought as a result of the deletion into the vicinity of the fetal γ -globin genes is responsible in some way for the continuing expression of γ -globin genes in HPFH. Characterization of the DNA at the 3' end point of these HPFH deletions is in progress and may elucidate further the mechanisms responsible for the HPFH phenotype.

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BOOK REVIEWS

The central problem in human evolution

C. Owen Lovejoy

DIET has always figured prominently in studies of the adaptation and evolution of mammals. This is to be expected, as most of the fossil record is composed of teeth and jaws, and feeding adaptations, aside from reproduction, are probably the most critical selective force in mammalian evolution. Moreover, the two-million-year archaeological record is made up of food remains and the tools used to obtain and process them. Superimpose upon this background such current debates as human sex roles, the quantitative analysis of archaeological sites and the forces at play in the origin of hominids, and the result is a cauldron of intense debate about facts and theories of almost every phase of human evolution.

The fourteen chapters comprising Omnivorous Primates are predominantly devoted directly to the problem of reconstructing the role of diet in human evolution. There are essentially four ways by which this can be done: by study of the archaeological record (three chapters); by study of the dietary habits of non-human primates (four chapters); by study of the dietary habits of contemporary huntergatherers (four chapters); and by direct study of the dental remains of hominids. Given the great advances and significant work which has been conducted in the past few years in the latter area, it is surprising that this volume contains no contributions dealing directly with these methods. One additional chapter which was not classified above deserves special mention. Alan Mann presents a thorough general essay on diet in the Plio-Pleistocene hominids using all of the four categories of data. He details dietary requirements of primates, reviews the historical development of various theories of feeding strategy in early human evolution (for example hunting, seedeating, gathering), looks at the direct evidence available in cranial morphology, and summarizes with a discussion of models of early hominid dietary exploitation, concluding that the best model is one which posits a diet of marked diversity, significantly enhanced by the use of primitive tools with which to exploit underground food sources. It is of interest that the diet posited by Mann (as he himself points out) is quite similar to that found in Gombe chimpanzees (minus the underground rhizomes).

In his own paper Teleki provides a detailed account of this diet, including data on activity rhythms and feeding budgets. Most of his contribution, however, is

Omnivorous Primates: Gathering and Hunting in Human Evolution. Edited by Robert S. O. Harding and Geza Teleki. Pp.673. ISBN 0-231-04024-5. (Columbia University Press: 1981.) \$52, £28.60. Woman The Gatherer. Edited by Frances Dahlberg. Pp.250. ISBN 0-300-02572-6. (Yale University Press: 1981.) \$15, £10.50.

devoted to a factual description of chimpanzee meat consumption, in which he notes many aspects which may bear significantly on early human behaviour (for example evidence of "cooperative behaviour", food sharing and a sexual division of activity, to wit: "pursuit, capture and consumption of animals are primarily male activities, while collection of insects . . . is primarily a female activity"). In fact he concludes that "chimpanzees are ecologically, behaviourally, socially, and psychologically best suited to serve as a model for reconstructing human origins".

There are a number of similarities between Teleki's chimpanzees and the hunting behaviour in Gilgil baboons which is carefully presented by Shirley Strum (including cooperation, sexual differences in hunting behaviour and food sharing), but her contribution is of particular note for a different reason. Her continuous observation of a single group for over seven years has allowed her to chronicle significant changes in behaviour in response to ecological, demographic and social fluctuations. Her report strikes home the too-often forgotten fact that our knowledge of primate behaviour is based on but a few short-term studies of single groups which must be extended to millions of years of primate evolution.

As a whole, the volume is well-edited and produced, and will be valuable not only to those interested in human evolution and primate behaviour, but archaeological methods and ethnography as well. All of the contributions are competent and informative. Harding presents a general review of primate diet, and Hayden a similar world-wide review of diet in modern hunter-gatherers. Gould's review of food-acquisition and sharing behaviour in Australia and north-west California should prove particularly useful to prehistorians who must deal with the problem of the interpretation of food remains from archaeological sites. There is an informative up-date on the age-old

question of Pleistocene extinctions by David Webster.

In contrast to Omnivorous Primates, Woman the Gatherer contains virtually no contributions to the current debate. The title is an obvious word-play with respect to an earlier volume which has had farreaching influence in modern anthropology, Lee and Devore's Man the Hunter. That volume was, in fact, more responsible than any other (despite some of its contributions and title to the contrary) in fully establishing the crucial role of women as gatherers and contributors to the success and function of pre-urban human societies. This new book, its title again to the contrary, contains little to amplify this now generally recognized fact. The article by McGrew, summarizing observations from a variety of field studies, is a thorough and highly competent account of (primarily female) chimpanzee behaviour, and in this regard is useful and informative. The model presented at the end of the article, however, falls short of actually being a model, but rather stands only as a brief account of a theoretical chimpanzeelike-hominoid to hominid transition without attention to selective mechanisms - the critical element in any successful model. A contribution by Zihlman, on the role of female gathering in human evolution, while again a useful account, suffers from the same deficiency.

Other papers in this volume, although of general interest, fall short of having a significant bearing on the actual role of females in long-term human evolution. Papers on hunting behaviour among Agta women, Mbuti womanhood, and the collection and preparation of food by Chipewayan women are useful contributions, but fall short of being directly applicable to the problems of human evolution. A long and involved paper by Catherine Berndt presents a rather cryptic account of the misuse of data obtained from observations of the Australian aborigines. Readers will suffer from her inherent assumption that they are intimately familiar with this arena of

These two volumes, taken as a pair, raise certain rather interesting questions and paradoxes about viewpoints frequently adopted in the study of human evolution. The most disturbing of these is the failure to recognize the relatively enormous behavioural "gulf" between the australopithecines and modern man. Based upon what we know of the morphology of these

two taxa, that "gulf" must be approximately similar in magnitude to present differences between chimpanzees and hunter-gatherers. Primatologists (and one sees it frequently in these two volumes) often adopt an attitude that human behaviour is little more than slightly elaborated chimpanzee (or other primate) behaviour; ethnographers can, to the contrary, take the view that australopithecines were hunter-gatherers with robust faces and walnut-sized brains. Both groups would do well to study with great care the contributions to Omnivorous Primates by Freeman and Klein. These authors carefully document and chronicle the dramatic and progressive development of dietary shifts which took place in the middle and late Pleistocene in the Iberian Peninsula and the circumcoastal region of South Africa. While the question of the actual origin of hominids is critical and intriguing, the transformation of a

chimpanzee-like bipedal hominid into a cognitive, social and technological animal took place during the middle Pleistocene, a period for which no living analogues exist. There has been an overall assumption that Homo erectus was simply an intermediate between australopithecine and human being, but the archaeological evidence does much to contradict such an assumption. All living societies, no matter how "primitive", post-date the dramatic advances in upper Palaeolithic technology and the unquestionably equally dramatic alterations in social and subsistence behaviour which accompanied them. It is clear that we will lack a complete understanding of the process of human evolution until this vast yet crucial middle period is given the detailed attention it most sorely deserves. П

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An atomy of the Origin of Species

L. Beverly Halstead

A Concordance to Darwin's Origin of Species, 1st Edn. Edited by Paul H. Barrett, Donald J. Weinshank and Timothy T. Gottleber. Pp.834. ISBN 0-8014-1319-2. (Cornell University Press: 1982.) \$38.50, £27.

How appropriate it is, in the year marking one hundred years since Darwin's death, that his best-known book should join that other great British institution the "Complete Works of William Shakespeare" to say nothing of the Bible. All students of evolution and Charles Darwin will be indebted to Cornell University Press for their concordance to the *Origin of Species*, first edition.

This concordance is perhaps the supreme monument to what a computer can do with a book and also to what university teachers can accomplish with the help of undergraduates — who, in this case, patiently

typed 834 pages of text at 86 lines to a page. Unlike the concordance to the Bible, where the full context is listed under each entry and one can obtain all the useful quotations without ever having to open the Bible itself, with the present work such an approach is not possible. Each entry listed is printed in the centre of the page with sufficient of the adjacent words to fill a single line of print, no more and no less. This means that the entries rarely make up a complete sentence, and if they are at the end of a sentence the following quotation may well be entirely irrelevant to the entry. Reference to the first edition itself, or rather a facsimile, is thus essential. The publishers of the facsimile, Harvard University Press, should be duly grateful.

Every student of evolution will wish to possess this concordance, but it must be stressed that for all its thoroughness the three editors found it necessary to suppress certain words, for example "the" 10,144 times, "of" 7265, "in" 3904, "to" 3563; "you" which occurred three times was also suppressed.

To my amazement, Darwin did not include a single mention of the aardvark. and zoological appeared only twice with single entries for zoologist and zoologists. Geological, geologists and geology together merited 128 entries, thus emphasizing the relative importance of these two disciplines in Darwin's eyes. Even the creationists are well catered for in that they can readily list the number of qualifying prepositions, nouns, adverbs and adjectives used. Insights into Darwin's relationship with other scientists of the period can also be extracted from this work. Charles Lyell is mentioned 27 times in such phrases as "Lyell's noble views" "Lyell's grand work", "Lyell's profound remark" "Lyell's manual will bring home the truth". Huxley rates a mere 4 mentions. Murchison 4, Adam Sedgwick 2, whereas Owen and Agassiz with 18 and 10 respectively do much better.

The use of certain words must surely be significant: for example Darwin uses the first person singular some 999 times. And although Darwin's theory arose primarily from his circumnavigation of the world aboard the H.M.S. Beagle, this vessel is mentioned but twice, firstly in the opening sentence of the book: "When on board H.M.S. Beagle, as naturalist, I was much struck by certai" (there the entry ends). The other word that is remarkable for the circumspection with which it was used by Darwin is the last word in the book: "and most wonderful have been, and are being, evolved".

There is one further criticism that can be levelled at this volume: at no place is it possible to discover the full title of Darwin's book. Once again it is necessary to refer to the Harvard facsimile: On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life.

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OA11 groups under groups. This classification is EVIDENTLY not arbitrary like the grouping of the star 2011 a fair balance be struck between the good and EVIL caused by each part, each will be found on the 3490 and most wonderful have been, and are being, EVOLVED.

OCOS 18 have pollen utterly worthless, in the same EXACT condition as in the most sterile hybrids. Whe could be seen that this is not so in more cases, in which EXACT records have been kept i thus, to give a very could be seen that this is not so in more cases, in which EXACT records have been kept into the stages of 0237 which on my theory we ought to find. Moreover, IF we look to rather wider intervals, namely, to do 1000 glons of the whole world in organic beings; yet IF all the species were to be collected which ever 10311 nat gradations between any two or more species. IF such gradations were not fully preserved, trans 10311 logists, be ranked as new and distinct species. IF then, there be some degree of truth in these re 10311 logists, be ranked as new and distinct species. IF numerous species, betonging to the same genera 10312 ucceeding formstion such species will appear as IF suddenly races of the same formation and 1030 in the stay are at present. Even at this day, IF the maley Archipelago were converted into land, 10307 acters in any degree intermediate between them. IF, roreover, they had been the progenitors of the 10307 userous and improved descendants. Consequently, IF my theory had been the progenitors of the 10307 userous and improved descendants. Consequently, IF my theory had been the progenitors of the 10307 intervened during these encreously long periods. IF them we may inter anything free these facts, we 10312 creat beds, though undoubtedly of high entituity IF measured by years, only one or two species are 10312 r, as far as we know, on the face of the sarth. IF we compare any but the most closely related for 10312 in the 1032 in the 1032 in the 1032 in the 1033 in the 1034 change
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Ion transport for enthusiasts

N. Michael Green

The Sarcoplasmic Reticulum: Transport and Energy Transduction. By Leopoldo de Meis. Pp.163. ISBN 0-471-05025-3. (Wiley:1981.) £29.25, \$52.50.

Over the past 12 years the sarcoplasmic reticulum has become increasingly popular as a model system for the study of transport of ions across membranes. Physiology, electron microscopy, techniques of membrane reconstitution, lipid chemistry, protein chemistry and enzyme kinetics have all contributed to make this one of the best known of subcellular organelles.

This short monograph is concerned with the author's own field of kinetic studies on isolated vesicles of sarcoplasmic reticulum, to which he has made significant contributions. After two concise and informative introductory chapters, Dr de Meis restricts himself to this area. By concentrating his attention in this way he is able to present a variety of steady state and transient state kinetic experiments which have led to the generally accepted minimal cycle of at least eight reversible reactions that are responsible for Ca++ translocation. Since Ca++, Mg++ and ATP each enter the cycle more than once, it has required considerable experimental ingenuity to provide even a qualitative description of the reaction sequence. The chief merit of the book is that it presents crucial experiments from laboratories in well-illustrated detail, providing a valuable perspective for anyone trying to find his way through the 300 or more papers quoted. Mastery of the evidence presented will bring the reader to the end of 1980 and to a good position from which to cope with the arguments of 1981 and beyond, for the story is by no means over.

The author says very little on several important questions on which the evidence is conflicting and it would have been helpful to have had these problems more clearly delineated. They include the related questions of a monomeric versus a dimeric functional unit and of the number of Ca⁺⁺ and ATP binding sites per peptide chain. The questions of co-transport or countertransport of other ions and of the possible electrogenic nature of the pump are barely considered, while excitation-contraction coupling and Ca⁺⁺ release mechanisms are explicitly excluded.

The final chapter on the role of water in the active site of the ATPase is rather speculative, being based in part on the effects on the stability of the acyl phosphate of dimethylsulphoxide, which is assumed to be a hydrophobic solvent; this assumption is hardly warranted by its very high dielectric constant. The remarkable changes in reactivity undergone by the acyl phosphate intermediate during the catalytic cycle are more likely to be a

consequence of local electronic influences than of a general change in accessibility of the catalytic site to water.

These are minor reservations, however, and do not seriously detract from the value of this book to all those interested in an analytical approach to ion transport.

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Intention in mind

Richard Gregory

Mind Design: Philosophy, Psychology, Artificial Intelligence. Edited by John Haugeland. Pp.368. Hbk ISBN 0-226-08110-5; pbk ISBN 0-226-58052-1. (MIT Press: 1981.) Hbk \$21.50, £13.50; pbk \$10, £6.20. Minds and Mechanisms: Philosophical Psychology and Computational Models. By Margaret A. Boden. Pp.311. ISBN 0-855-27064-0. (Harvester/Cornell University Press: 1981.) £20, \$29.50.

Mind Design, a collection of 12 papers by computer scientists and philosophers of AI, was conceived by its editor, John Haugeland, as a sequel to A.R. Anderson's Minds and Machines which was published by Prentice-Hall in 1964. This new collection offers essays by Allen Newell and Herbert Simon; Zenon Pylyshyn; Marvin Minsky; Drew McDermott; Hubert Dreyfus; Hilary Putnam; Daniel Dennett; John Searle; Jerry Fodor; and Donald Davidson. There is also the particularly welcome essay, "Artificial Intelligence - A Personal View", by David Marr, whose recent death while at the height of his genius was a tragic blow for computer vision as well as for his many friends. The editor himself contributes an insightful Introduction, "Semantic Engines", and a rather less interesting essay on cognitivism.

In his Introduction, Haugeland asks how the psychology of thought can be properly scientific when thoughts cannot be observed; though the problem is not like that of galaxies or electrons, that they are too far away or too small. Referring to the "marvellously rich analogy with computers", and especially to a chessplaying machine, which "would be awkward to describe [in] its functioning by assigning geometrical shapes and locations to the internal program routines, yet has no immaterial soul" the crucial question is raised: how far is the human chess player like the machine that may beat him in intellectual combat?

A central issue throughout the book is



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The Changing Climate:

Responses of the Natural Flora and Fauna

Michael J. Ford.

Nature Conservancy Council

Choosing a wide range of natural examples, Dr Ford considers the different levels at which climatic effects operate on individual species and communities. Although the magnitude of climatic change this century is quite small, the author argues that its effect on natural flora and fauna may be disproportionate. Man's destruction of natural habitats includes climatically favourable areas providing refuge in times of climatic stress. If these disappear, certain species may be endangered.

April 1982 190 pp 0 04 574017 8 Hardback £13.95/\$27.50

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intention; here the American philosopher Dan Dennett has interesting things to say, also using the chess-playing computer example. He points out that even the designer cannot predict the next move of the machine from its design. He can only do so by considering rational and good moves of the game, which implies that the machine "chooses" rational and good moves. So, one is viewing machines such as these in intentional terms. Dennett, however, is careful to say that we may be right to attribute a lot more to human beings than this rather limited kind of intention, though he is not sure what this extra is.

Hubert Dreyfus is the respected devil's advocate of AI, whose book What Computers Can't Do (Harper & Row, 1972) provided, to the credit of all, reasoned criticisms that have been discussed with reason and not merely dismissed by the high priests of AI. Here Dreyfus adapts to the new developments of computer systems, starting with the universally acknowledged achievement of Winograd's natural language understanding program SHRDLU of 1972, which worked in a simplified micro-world, which was not much like our reality. Dreyfus objects to a common AI assumption, that such manageable microworlds can be generalized to the real world of physics and people. He suggests that proponents of AI are misled by the success of test-tube procedures in the natural sciences into thinking that these microworlds are even relevant; because although the test-tube worlds of physics obey physical laws, the computer micro-worlds may obey only their own laws. I suppose the answer is that "mental" laws are not physical laws anyway, so the whole exercise is outside physics; but it may still be science. Perhaps only physicists will strongly object to this conclusion.

Marvin Minsky invokes the notion of structures of knowledge — "frames" — to match common situations and to be adapted to the special features of situations. He attributes human intelligence to the individual's ability to select frames appropriately and rapidly. Selected knowledge frames are adapted to match the details of particular situations as they are encountered. This bears a resemblance to Bartlett's "Schemas" for locking together and giving significance to perceptions and memories. It is also related to David Marr's "Primal Sketch", his

AI at MIT

At the end of April, MIT Press will publish a two-volume paperback edition of Artificial Intelligence: An MIT Perspective edited by Patrick Henry Winston and Richard Henry Brown. The original hardback edition was reviewed in Nature 282, 540; 1979. Price of the paperback version is \$12.50, £8.75 per volume; hardback is available at \$25, £17.50 per volume.

initial descriptive stage of visual processing.

Several of the authors here express doubts on the scientific status of AI and its claims and results. For Marr,

a result in AI consists of the isolation of a particular information-processing problem, the formulation of a computational theory for it, the construction of an algorithm that implements it, and a practical demonstration that the algorithm is successful.

He points out, though, that there may be nice and neat successful solutions (which may not be found) and also messy solutions which may be as successful. Given the aesthetics of science, one may guess that AI will be accepted as properly scientific only if its solutions are both successful and elegant, whatever be the case for our own brain's functions — even when we are thinking about the challenging intellectual issues presented in these illuminating essays on artificial intelligence.

Margaret Boden's Minds and Mechanisms is a collection of her own papers on the philosophy of AI. Her concern with the concept of intention goes back to her first book, Purposive Explanation in

Psychology (Harvard University Press, 1972), and is also a theme of her widely acknowledged text, Artificial Intelligence and Natural Man (Harvester, 1977). Intention appears to be basic to any language system, and Professor Boden was largely responsible for pointing this out. Her strength lies in combining a deep knowledge of cognitive psychology, and what AI programs can and cannot at present do, with a background in both natural science and philosophy. The result is an authoritative book, though inevitably there is unevenness and some redundancy as the papers were written separately and for somewhat different audiences. Some of them take us more deeply into the issues of her previous books, and the technical detail presented here justifies careful reading for appreciating the fascination of mind struggling to be transmigrated into manmade machines that surprise and puzzle

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Polymer dynamics revealed

F.A. Bovey

Molecular Motion in High Polymers. By R.T. Bailey, Alastair M. North and Richard A. Pethrick. Pp.415. ISBN 0-19-851333-X. (Clarendon/Oxford University Press: 1981.) £42.50, \$69.

As they state in their preface, in Molecular Motion in High Polymers the authors have essentially produced two books under one cover. Part A is primarily theoretical in nature and, after a general introduction, deals with normal vibrations and inelastic light scattering, various "hydrodynamic" models of chain motion and statistical theories of polymer molecular motion. The level of mathematical treatment is moderately high, and readers not comfortable with vector and tensor notation and matrix algebra will find themselves somewhat at sea.

Part B is described as a phenomenological treatment. It is more descriptive and the mathematical background is less prominent, although not at an elementary level. Most readers should be able to absorb this section with profit without necessarily resorting to Part A. Dielectric properties, photoluminescence, viscoelastic relaxation, ultrasonic relaxation, nuclear magnetic and electron spin resonance, spectroscopic and scattering phenomena, neutron scattering and diffusion are all well described.

My reservations about the book mainly concern the completeness of the treatment. More specifically, the literature citations

have a dated appearance. It should be possible to reference work within at most two years of the publication date, and in fact there is a reference to a 1980 paper by one of the authors. However, in general there are few references to papers later than 1975. This may be acceptable in an introductory textbook, but not in a scholarly treatise such as this. I was especially sensitive to this point in the chapter on nuclear magnetic and electron spin relaxation, my own field. Carbon-13 NMR, the area of greatest recent interest, is given rather short shrift. Although highresolution carbon-13 NMR of solids is described, the treatment is very compressed. Carbon-13 relaxation in solution, a major area, occupies less than a page, and most of the relevant literature is omitted. It should also be pointed out that the section on the inversion-recovery method for measuring T, has been mislabelled "Carr-Purcell Experiments", a quite different technique used for measuring T2.

This selective treatment means that readers cannot count on being brought to the forefront of the topics described. Despite this reservation, I found the discussion informative and valuable. The book is well written and can be read with pleasure.

F.A. Bovey is Head of the Polymer Chemistry Research Department, Bell Laboratories, Murray Hill, New Jersey.

BOOKS RECEIVED

Earth Sciences

AGER, D.V. The Nature of the Stratigraphical Record. 2nd Edn. Pp.122. Pbk ISBN 0-470-27052-7. (Macmillan Press, London and New York: 1981.) Pbk £5.50. FAUST, S.D. and ALY, O.M. Chemistry of Natural Waters. Pp.399. ISBN

0-250-40387-0. (Ann Arbor Science/Butterworths: 1981.) £23.10.

LEATHERMAN, S.P. (ed.). Overwash Processes. Benchmark Papers in Geology, Vol.58. Pp.376. ISBN 0-87933-375-8. (Academic: 1981.) \$45.

POUGH, F.H. A Field Guide to Rocks and Minerals. 4th Edn. Pp.317. Hbk ISBN 0-395-08106-8; pbk ISBN 0-395-24049-2. (Constable, London: 1981.) Hbk £5.95; pbk np.

WHITMORE, T.C. (ed.). Wallace's Line and Plate Tectonics. Pp.90. ISBN 0-19-854545-2. (Clarendon Press/Oxford University Press: 1981.) £15.

Biological Sciences

BEERS, R.F. Jr. and BASSETT, E.G. (eds). Nutritional Factors: Modulating Effects on Metabolic Processes. Miles International Symposium Series, Number 13. Pp.582. ISBN 0-89004-592-5. (Raven: 1981.) \$55.

BLOCH, K., BOLIS, L. and TOSTESON, D.C. (eds). Membranes, Molecules, Toxins, and Cells. Pp.315. ISBN 0-88416-309-1. (John Wright, Bristol: 1981.)

BLYSKOWSKI, A.H. The Discovery of Cancer Enigma. Pp.190. Pbk ISBN 0-9507552-0-6. (Scientific Press, Kenton: 1981.) Pbk £6.

BROOM, D.M. Biology of Behavior. Mechanisms, Functions and Applications. Pp.320. Hbk ISBN 0-521-23316-X; pbk ISBN 0-521-29906-3. (Cambridge University Press: 1981.) Hbk £20; pbk £7.95.

BROWN, A.G. Organization in the Spinal Cord. The Anatomy and Physiology of Identified Neurones. Pp.238. ISBN 3-540-10549-2. (Springer-Verlag: 1981.) DM 170, \$77, 30.

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DINGLE, J.T. and GORDON, J.L. Cellular Interactions. Research Monographs in Cell and Tissue Physiology, Vol.6. Pp.326. ISBN 0-444-80330-0.

(Elsevier Biomedical: 1981.) \$80, Dfl.188.

DOWBEN, R.M. and SHAY, J.W. (eds). Cell and Muscle Motility, Vol.1.
Pp.400. ISBN 0-306-40703-5. (Plenum: 1981.) \$30.50.

EMMERS,R. Pain: A Spike-Interval Coded Message in the Brain. Pp.144. ISBN 0-89004-650-6. (Raven: 1981.) \$25.

ENNA, S.J., SAMORAJSKI, T. and BEER, B. (eds). Brain Neurotransmitters and Receptors in Aging and Age-Related Disorders. Aging Series, Vol.17. Pp.292.

ISBN 0-89004-643-3. (Raven: 1981.) \$32. GERSHWIN, M.E. and MERCHANT, B. (eds). Immunologic Defects in Laboratory Animals, 2. Pp.382. ISBN 0-306-40673-X. (Plenum: 1981.) \$42.50.

GETTY, D.J. and HOWARD, J.H. Jr. (eds). Auditory and Visual Pattern Recognition. Pp.222. ISBN 0-89859-087-6. (Lawrence Erlbaum, London: 1981.)

GIBBS, E.P.J. (ed.). Virus Diseases of Food Animals. A World Geography of Epidemiology and Control. Vol.I, International Perspectives. Pp.330. ISBN 0-12-282201-3. (Academic: 1981.) £28, \$57.50.

HEINZ, E. Electrical Potentials in Biological Membrane Transport. Molecular Biology, Biochemistry and Biophysics, Vol.33. Pp.85. ISBN 3-540-10928-5. (Springer-Verlag: 1981.) DM 69, \$29.40.

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HOKE, M. and WIGAN, M.E. (eds). Sudden Loss of Cochlear and Vestibular Function. Advances in Oto-Rhino-Laryngology, Vol.27. Pp.198. ISBN 3-8055-2630-X. (Karger, Basel: 1981.) SwFr. 148, DM 177, \$88.75.

HOLCOMB, D.N. and KALAB, M. (eds.). Studies of Food Microstructure. Based on Programmes Organized by S.H. Cohen et al. Pp.342. ISBN 0-931288-22-3. (Scanning Electron Microscopy, Inc., Illinois: 1981.) Np.

HORECKER, B.L. and STADTMAN, E.R. (eds). Current Topics in Cellular Regulation, Vol.19. Pp.324. ISBN 0-12-152819-7. (Academic: 1981.) \$39.

HURLBERT, S.H. (ed.). Aquatic Biota of Southern South America being a Compilation of Taxonomic Bibliographies for the Fauna and Flora of Inland Waters of Southern South America. Pp.342. (no ISBN) (San Diego State University: 1981.) Pbk \$8 (in Latin America); \$13 (elsewhere).

HURLBERT, S.H., RODRIGUEZ, G. and DOS SANTOS, N.D. (eds).

Aquatic Biota of Tropical South America being a Compilation of Taxonomic Bibliographies for the Fauna and Flora of Inland Waters of the Tropical Portion of South America. Part 1, Arthropoda. Pp.323; Part 2, Anarthropoda. Pp.298. (San Diego State University: 1981.) Part 1 pbk \$14; part 2 pbk \$12.

INTERNATIONAL ASSOCIATION OF BIOLOGICAL STANDARDIZATION (eds). International Symposium on Fish Biologics. Vol.49, Developments in Biological Standardization. Pp.496. ISBN 3-8055-3471-X. (Karger, Basel: 1981.)

SwFr. 94, DM 113, \$56.50.

JACKSON, W.P.U. Wild Flowers of the Fairest Cape. Pp.126. ISBN 0-86978-194-4. (Robert Hale, London: 1982.) £15.

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LEWIN, R.A. The Biology of Algae and Other Verses. Pp.103. Pbk ISBN 0-8191-1689-0. (University Press of America, Washington: 1981.) Pbk \$6.75.

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March 1980. Pp.355. ISBN 0-89004-597-6. (Raven: 1981.) \$25.50.
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General

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POWER, J. Amnesty International: The Human Rights Story. Pp.128. ISBN 0-08-028902-9. (Pergamon: 1981.) £8.75, \$17.50.
PRICE, L.W. Mountains and Man: A Study of Process and Environment.

Pp.506. ISBN 0-520-03263-2. (University of California Press: 1981.) £21, \$37.45.

RADFORD, A.E. et al. Natural Heritage. Classification, Inventory, and Information. Pp.485. ISBN 0-8078-1463-6. (The University of North Carolina Press: 1981.) \$25.

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SKINNER, B.F. Notebooks. Pp.386. ISBN 0-13-624106-9. (Prentice-Hall: 1981.) £11.95.

SLADEK, F.E. and STEIN, E.L. Grant Budgeting and Finance. Getting the Most Out of Your Grant Dollar, Pp.328, ISBN 0-306-40607-1. (Plenum: 1981.) \$19.50.

ANNOUNCEMENTS

Awards

Professor George Weber (Laboratory for Experimental Oncology at Indiana University School of Medicine) has been elected for the 22nd Annual Clowes Award by the American Association for Cancer Research.

The Institution of Geologists has presented the Aberconway Medal to Dr D.J. Burdon.

Appointments

Dr David Mahoney is to head CSIRO's new Division of Tropical Animal Science.

Dr Robert E. Olson has been appointed associate dean for academic affairs at the University of Pittsburgh School of Medicine and also professor in the Departments of Biochemistry and Medicine.

James "Scotty" Miller has been named the first Director of Methods Development for the American Oil Chemists' Society.

Royal Society Fellows

At a meeting of the society on 18 March 1982, the following were elected Fellows of the Royal Society: Dr Ulrich Wolfgang Arndt (Medical Research Council Laboratory of Molecular Biology. Cambridge). Professor Rodney James Baxter (Theoretical Physics, Australian National University, Canberra); Professor Michael Victor Berry (Theoretical Physics, University of Bristol); Professor James Derek Birchall (ICI Ltd, Runcorn, Cheshire, and visiting professor of Materials Science, University of Surrey); Professor Anthony David Bradshaw (Botany, University of Liverpool); Dr Daniel McGillivray Brown (Organic Chemistry, University of Cambridge); Dr Lawrence Michael Brown (Physics, University of Cambridge); Professor Bryan Campbell Clarke (Genetics, University of Nottingham); Professor William Maxwell Cowan (Anatomy, Salk Institute for Biological Studies, San Diego, USA); Professor Alan William Cuthbert (Pharmacology, University of Cambridge); Dr John Thomas Finch (Medical Research Council Laboratory of Molecular Biology. Cambridge); Professor Henry Edgar Hall (Physics, University of Manchester); Professor Michael Hart (Physics, King's College, University of London); Dr Eric John Hewitt (Plant Physiology and Biochemistry Section of Long Ashton Research Station, Bristol, and Plant Physiology and Biochemistry, University of Bristol); Professor Charles Antony Richard Hoare (Computation, University of Oxford); Professor Robert Francis Hudson (Organic Chemistry, University of Kent at Canterbury); Professor Kenneth Langstreth Johnson (Engineering, University of Cambridge); Professor William

Johnson (Mechanics, University of Cambridge); Professor Peter Julius Lachmann (Tumour Immunology, University of Cambridge and Medical Research Council Unit on Mechanisms in Tumour Immunity, Cambridge); Dr Ralph Lainson (Wellcome Parasitology Unit, Belém, Brazil): Dr Michael Francis Land (Biological Sciences, University of Sussex); Professor Peter John Lawrenson (Electrical and Electronic Engineering, University of Leeds); Professor William Russell Levick (Physiology, John Curtin School of Medical Research, Australian National University, Canberra): Professor Stephen Finney Mason (Chemistry, King's College, University of London); Dr Noreen Elizabeth Murray (Molecular Biology, University of Edinburgh); Sir Gustav Nossal (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, and Medical Biology, University of Melbourne); Professor Ronald Harry Ottewill (Colloid Science, University of Bristol); Dr William James Peacock (Division of Plant Industry, CSIRO, Canberra, Australia); Professor Philip James Edwin Peebles (Physics, Princeton University, USA); Peter Rainger (British Broadcasting Corporation, London); Professor Chintamani Nagesa Ramachandra Rao (Indian Institute of Science, Bangalore); Dr Lewis Edward John Roberts (Atomic Energy Research Establishment, Harwell, and Board of the United Kingdom Atomic Energy Authority); Professor George Stanley Rushbrooke (Theoretical Physics, University of Newcastle upon Tyne); Professor John George Sclater (Marine Geophysics, Massachusetts Institute of Technology, USA); Dr Graeme Bryce Segal (Mathematics, University of Oxford); Professor David Rostron Trentham (Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, USA); Professor John Stewart Turner (Geophysical Fluid Dynamics, Australian National University, Canberra); Professor Thomas Gaskell Tutin (Taxonomy, University of Leicester); Professor John Conrad Waterlow (Human Nutrition, London School of Hygiene and Tropical Medicine, University of London).

Meetings

16-18 May, Biomedical Applications of LCEC and Voltammetry, Indianapolis (LCEC Symposium, PO Box 2206, West Lafayette, Indianapolis 47906, USA).

17-18 May, Cancer, London (The Marie Curie Memorial Foundation, 124 Sloane St, London SW1, UK).

17-21 May, Applied Combustion Technology, Geneva (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

18-20 May, Roots at Work, Wantage (Dr R.S. Bruce, ARC Letcombe Laboratory, Wantage, Oxon, UK).

19 May, Landfall Leachate Symposium, Oxford (Mr L. Evans, Education and Training Centre, Harwell Laboratory, Didcot, Oxon, UK).

24-27 May, Ceramics Technology for the Non-Ceramist, Amsterdam (The Centre for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

24-26 May, Male Transmission of Adverse Reproductive Effects, Cincinnati (Marvin Legator, University of Texas, Galveston, Texas 77550, USA).

25-28 May, **Biological Basis of New Developments**, Minnesota (P. Rogers, 1060, Mayo Memorial Bldg, Box 196, 420 Delaware St, SE Minneapolis, Minnesota 55455, USA).

24-28 May, Applied Pump Technology, Amsterdam (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

24-28 May, CAgM Working Group on Weather and Animal Health, Geneva (Research and Applications Programme Dept, World Meteorological Organization, Geneva, Switzerland).

25 May, Chemistry which Imitates Biochemistry: Artificial Enzymes, London (Miss J. Holman, The Ciba Foundation, 41 Portland Place, London W1, UK).

2-4 June, International Chlorine Symposium, London (The Conference Secretariat, Society of Chemical Industry, 14/15 Belgrave Square, London SW1, UK).

2-4 June, Endocrine Lung in Health and Disease, Washington DC (P. J. Petrossian, GWUMC-Office of CME, 2300 K. St, N.W. Washington DC 20037, USA).

13-16 June, **Birth Defects Conference**, Alabama (March of Dimes, 1275 Mamaroneck Ave, White Plains, New York 10605, USA).

29-June-1 July, Crystallization Technology, Amsterdam (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

20 June-3 July, The Application of Laser Light Scattering to the Study of Biological Motion, Maratea (Charles Barker Lyons Ltd, 30 Farringdon St, London EC4, UK). 21-25 June, Radioimmunoassay and Related Procedures in Medicine, Vienna (IAEA, Wagramerstrasse 5, PO Box 100, A-1400 Vienna, Austria).

21 June-3 July, Cancer Epidemiology, Jabonna (International Agency for Research on Cancer, 150 cours Albert Thomas, F-69372 Lyon Cedex 08, France). 30 June-2 July, Applications of Vibrational Spectroscopy, Southampton (Dr J. Evans, Dept of Chemistry, The University, Southampton, UK).

- 5-9 July, Mechanisms of Reactions in Solution, Canterbury (Dr J. F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).
- 8-9 July, Materials and Testing London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).
- 12-14 July, Electronic Systems Effectiveness and Life Cycle Costing, Norwich (Charles Baker Lyons Ltd, 30 Farringdon St, London EC4, UK).
- 17-25 July, Microbiology, Kansas (Dr Fung, Call Hall, Kansas State University, Manhattan, Kansas 66506, USA).
- 19-23 July, Statistical Methods in Cancer Epidemiology, Lyon (International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 8, France).
- 19-23 July, Correlation Analysis in Organic Chemistry, Hull (Dr J. F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).
- 19-24 July, European Congress on Cell Biology, Paris (Ist European Congress on Cell Biology, 67, rue Maurice-Günsbourg, 94200 Ivry-sur Seine, France).
- 19-30 July, Air-Sea Exchange of Gases and Particles, Durham USA (Charles Barker Lyons Ltd, 30 Farringdon St, London EC4, UK).
- 27-29 July, Strategies for Coping with Critical Issues Related to Materials and Minerals, New Hampshire (FMS, 345 East 47th St, New York, New York 10017, USA). 26 July-6 August, Genetic Engineering in Eukaryotes, Pullman (Charles Barker Lyons Ltd, 30 Farringdon St, London FC4, UK).
- 31 July-6 August, Living with Industry, West Yorks, (The SISCON Summer School Secretary, Combined Studies in Science, University of Leeds, Leeds, UK). 1-11 August, 11th INQUA Congress, Moscow (Prof. Dr Marton Pecsi, Institute of Geography, Academy of Sciences, Budapest, Hungary).
- 2-5 August, Meeting of the American Society for Viriology, Ithaca (H. S. Ginsberg, Dept of Microbiology, College of Physicians and Surgeons of Columbia University, 701 West 168th St, New York, New York 10032, USA).
- 2-6 August, Applications of X-ray Analysis, Denver (Mrs M. Cain, Denver Research Institute, University of Denver, Colorado 80208, USA).
- 5-6 August, Computational Physics on the Distributed Array Processor, Glasgow (The Institute of Physics, 47 Belgrave Square, London SW1, UK)
- 8-13 August, 9th Congress of the International Primatological Society, Atlanta (Dr F. King, Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30322, USA). 9-13 August, Joint Meeting, Electron Microscopy Society and Microbeam Analysis Society, Washington DC (Prof. T.E. Mitchell, Dept of Metallurgy and Materials Science, Case Western Reserve University, Cleveland, Ohio 44106, USA).

- 15-17 August, Functional Properties of Cereal Carbohydrates, Winnipeg (Dr R.D. Hill, Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2). 16-18 August, Potential Utility of NMR in Research and Clinical Applications, Boston (Dr M.R. Goldman, Internal Medicine, Massachusetts General Hospital,
- 18-22 August, SV40, New York (G. Kist, Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724, USA).

Boston, Massachusetts 02114, USA).

- 15-19 August, Genetic Engineering of Plants, California (Tsune Kosuge, Dept of Plant Pathology, University of California, Davis, California 95616, USA).
- 16-29 August, New Developments and Methods in Membrane Research and Biological Energy Transduction, Spetsai (Prof., Dr K.W.A. Wirtz, State University of Utrecht, Laboratory of Biochemistry, NL-3508 TB, The Netherlands).
- 24-29 August, **Phage**, New York (G. Kist, Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724, USA).
- 30 August-2 September, Planetary Rings, Toulouse (CNES, Dept des Affaires Universitaires, 18, ave Edouard-Belin, 31055 Toulouse Cedex, France).
- 31 August-5 September, **Herpesvirus**, New York (G. Kist, Cold Spring Harbor Laboratory, New York 11724, USA).
- 31 August-2 September, Very Large Baseline Interferometry Techniques, Toulouse (CNES, Dept des Affaires Universitaires, 18 ave Edouard-Belin, 31055 Toulouse Cedex, France).
- 1-3 September, Annual Symposium of the Uranium Institute, London (The Uranium Institute, 8th Floor, New Zealand House, Haymarket, London SW1, UK).
- 1-3 September, Environment and Safety Conference, Wembley (Labmate Ltd, Newgate Sandpit Lane, St Albans, Herts, UK).
- 1-4 September, Mutihormonal Cellular Regulations in Neuroendocrinology, Colmar (Ph. Philippe Richard, Laboratoire de Physiologie Générale, Université Louis Pasteur, 21 rue René Descartes, 67084, Strasbourg Cedex, France).
- 6-10 September, **BA '82**, Liverpool (W. McQuarrie, University of Liverpool, Senate House, Abercromby Square, PO Box 147, Liverpool, UK).
- 8-9 September, Vertebrate Palaentology, London (Geological Society, Burlington House, London W1, UK).
- 8-10 September, **Optics** '82, Edinburgh (Institute of Physics, 47 Belgrave Square, London SW1, UK).
- 8-15 September, International Cancer Congress, Seattle (Congress Operations Office, 4th and Blanchard Building, Suite 1800, Seattle, Washington 98121, USA).
- 8-10 September, Coagulation, Cancer and Inflammation, Virginia (Dr A. P. Ball, National Heart, Lung and Blood Institute,

- Federal Building, Bethesda, Maryland 20205, USA).
- 8-18 September, Principles and Methods in Receptor Binding, Urbino (Dr I. Ceserani, Institute of Pharmacology and Pharmacognosy, University of Milan, Via A. del Sarto, 21, 20129 Milan, Italy).
- 9-10 September, Volcanic Processes in Marginal Basins, Keele (Geological Society of London, Burlington House, London W1, UK).
- 12-16 September, Loss Prevention and Safety Promotion in the Process Industries, Harrogate (The Institution of Chemical Engineers, George E. Davis Building, 165-171 Railway Terrace, Rugby, UK).
- 13-15 September, Supercritical Fluids: Their Chemistry and Applications, Cambridge (Dr W.R. Ladner, National Coal Board, Stoke Orchard, Cheltenham, Gloucester, UK).
- 13-16 September, 1982 Meteoretical Society, St Louis (Prof. Ghislaine Crozaz, Washington University, Box 1105, St Louis, Missouri 63130, USA).
- 13-17 September, The Neutron and its Applications, Cambridge (The Institute of Physics, 47 Belgrave Square, London SW1, UK).
- 13-17 September, International Society for Photogrammetry and Remote Sensing, Toulouse (GDTA, 18 ave Edouard-Belin, 31055 Toulouse Cedex, France).
- 13-17 September, 12th Congress of the International Federation of Societies of Cosmetic Chemists, Paris (Societe francais de Cosmetologie, 44 rue du 22 Septembre, 92400 Courbevoie, France).
- 13-17 September, World Filtration Congress, Philadelphia (The Filtration Society, 2 Woodstock Rd, Croydon, Surrey, UK).
 13-17 September, Chromatography,
- 13-17 September, Chromatography, London (Chromatography Discussion Group, Trent Polytechnic, Burton Street, Nottingham, UK).
- 19-25 September, Photosynthetic Prokaryotes, Bordeaux (G. Stanier, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France).
- 14-16 September, Neurophysics, Cambridge (Dr G. Leng, ARC Institute of Animal Physiology, Babraham, Cambridge, UK).
- 15-17 September 1st Meeting of the British Liquid Crystal Group, Southampton (Dr J.W. Emsley, Dept of Chemistry, The University, Southampton, UK).
- 20-24 September, Carbon '82, London (Society of Chemical Industry, 14/15 Belgrave Square, London SW1, UK).
- 20-24 September, Antibiotics and Antibiosis in Agriculture, Nottingham (Prof. M. Woodbine, University of Nottingham, Faculty of Agricultural Science, Sutton Bonington, Loughborough, Leicester, UK).
- 20-24 September, 10th Molecular Crystal Symposium, Quebec (Dr D. Chartrand, National Research Council of Canada, Ottawa, Canada K1A OR6).

nature

Does biotechnology have a price?

The five United States universities represented at the meeting to consider the commercialization of genetic manipulation have issued a statement whose omissions are more significant than its content.

Last week's gathering of five university presidents and their entourages at Pajaro Dunes in California was a predictably civilized occasion it would seem, but hardly productive. The communique afterwards put out (see Nature 1 April, p.381) delicately acknowledges that the interest of universities in the commercial exploitation of genetic manipulation may occasion difficulties; makes a few general observations to the effect that universities should not let corporate or individual relationships with external commercial interests interfere with teaching and research; says that universities holding patents should be free occasionally to award exclusive licences; and concludes that it will in most circumstances be unwise for universities to invest financially in companies in which faculty members have a direct and substantial interest.

These unremarkable observations are offered as a contribution to what the five presidents rightly expect will be a continuing discussion of the propriety of arrangements between universities and external commercial interests. Because groups of university presidents are free to spend a few days at the seaside in each other's company without issuing an account of what they talked about, the Pajaro Dunes statement is something to be grateful for. Indeed, the fact of the meeting implicitly and the communique explicitly acknowledge that the public interest in the commercialization of academic research overrides the interests of individual universities. So why does the communique fail even to mention the immediate and perplexing issues with which universities must grapple in the years ahead?

Take secrecy. One of the most obvious consequences of the interpenetrating networks of commercial agreements being forged with external commercial interests by institutions, departments and individual academics is that the free flow of ideas within laboratories will be inhibited by the fear that to say too much will be to hazard a commercial advantage. The Pajaro Dunes communique acknowledges the risk, and says that agreements with external interests should be "so constructed as not to promote a secrecy that will harm the progress of science. . ." But how, in the real world, is that to be accomplished?

What happens, for example, if two members of the same academic department are also the prime movers in two separate commercial companies? Or if two close colleagues are consultants for competing commercial concerns? Or if the research carried out by one group within a laboratory is supported by a commercial organization by means of an agreement that gives the company first refusal of patent rights? In each case (and others), close colleagues will be tempted to bite off their tongues for fear of providing clues revealing of the commercial interests of their sponsors. Previously innocent academics, probably less skilled than professional consultants at distinguishing between proprietary and general information, are likely to behave like clams even in discussions of such general questions as the mechanisms by which eukaryotic genes are regulated. For the time being, in any case, (the kinds of questions that commercial companies are likely to be asking of those who work for them as consultants.) But in most universities and academic departments, the tongues of externally-interested academics will be further tied by the knowledge that they cannot know which of their colleagues is working privately for which competing organization.

Disclosure of external interests is therefore an essential part of

the process of preserving the civility of those parts of the academic research enterprise recently discovered to be commercially important. But disclosure to whom? And to what extent? Will it suffice that the chairman of a department should know for whom his immediate colleagues are moonlighting? Will chairmen in their turn shoulder the responsibility implied in the confidentiality of the disclosures made to them of making sure that putatively competing academics are aware of each others' outside interests? Or might it be better that disclosure should be more general? And is there not then a danger that students would be apprised of information that would be an excuse for occupying the departmental chairman's office on some sunny afternoon when there is nothing else to do? It is no wonder that the participants in the meeting at Pajaro Dunes steered clear of questions such as these in their published statement. Questions such as these are nevertheless prominent among those universities that must decide for themselves in the next few weeks or months.

The Pajaro Dunes communique would have been more helpful if it had openly acknowledged that problems such as these exist. It should also have recognized that the difficulties occasioned by biotechnology differ only in degree from those with which universities have learned, however uneasily, to live. For biotechnology, the commercial exploitation of genetic manipulation differs for the time being from more familiar branches of technology only in the speed with which a bright idea may be translated into a patentable invention. In other fields, chemical engineering for example, the process of innovation consumes more time and is less certain. But it is well understood that much of the research carried out in university laboratories, in computer science for example, has led in recent years to important industrial improvements and, as a consequence, to a close interdependence between academics and industrial partners. The result, especially in the United States, is that there is now a modest army of academics acting as consultants to industrial companies while agreements between companies and academic laboratories for the support of basic science in return for beneficial treatment in the exploitation of innovations are by no means unprecedented or

From this it follows that universities cannot hope to devise rules for the proper exploitation of basic research in genetic manipulation unless these also apply to the regulation of other branches of technology. It is, however, plain that in biotechnology, more explicit rules for deciding how academics and academic departments should conduct themselves are necessary. Within departments, seemly rules about disclosure are plainly needed if academics are to continue to talk to each other without restraint. For universities, it is also essential that the basis on which agreements for the support of research by outside companies should be publicly disclosed. Failure in this respect will put in hazard public trust in the whole of higher education. And universities must more zealously than at present ensure that the education of students, graduate students in particular, is not distorted by the commercial extramural interests of those who are supposed to teach them. If this implies that the research projects on which graduate students are engaged should not be determined exclusively by individual members of a university faculty, the restriction of individuals' academic freedom that would imply would be a small price to pay for the preservation of the

reputation of universities to put the interests of their students first. Individual universities may not be too hard-pressed to devise rules for regulating what happens in departments with an interest in biotechnology. The difficulty will be to ensure that these can be applied across the board.

Some thought should also be given to the question of why the commercialization of basic research has recently thrown up so many problems. Government support for basic research is shrinking everywhere, while universities are being urged to look to industry for sponsorship. Universities and individual academics are being driven into the arms of those companies farsighted enough to recognize that they have much to gain from partnerships like these. There is much to be said for finding ways of turning academic discoveries into prosperity for the wider community. But while some academics appear genuinely to be excited by the challenge of turning their bright ideas into business enterprises, others appear to be at least as much intrigued by the prospect of monetary rewards. Explanations, or excuses, are not hard to find. Compared with their students who make a success in industry, academics are scandalously paid, for example. But has the traditional loyalty of academics to the institutions which employ them been eroded? And if so, what will be the consequences of that? It is a fair guess that the university presidents who met at Pajaro Dunes two weeks ago would have been fully conscious of this disturbing thought.

The Falkland War

The British Government hopes that diplomacy will avoid a war with Argentina. How?

The next few days are bound to have an air of unreality not merely for those who live and work in Britain or Argentina but for the rest of the world. A small British naval force is sailing to the South Atlantic with the intention of liberating the Falkland Islands from their occupation by an Argentinian force last weekend. The military task is formidable, for it is bound to be more difficult to dislodge an occupying force than it would have been to prevent it from landing in the first place. So too is the diplomatic task, for the government of Argentina has repeatedly declared its intention of staying put. Sensibly, however, the British government has promised to use the next few days for seeking some kind of solution. What might this be?

The facts are clear. There is no impartial body of opinion that disputes the illegality of what the Argentinian government has done. Moreover, the doctrine advanced in Buenos Aires that the Falkland Islands belong to Argentina because of their proximity is a dangerous doctrine, as the case of Ulster in Ireland shows. The Argentinian action in the Falkland Islands might have been seen in a different light if the 1,800 inhabitants of the islands had shown the slightest inclination to opt for Argentinian rather than British citizenship, and if Britain made no use of the territory (which serves as a staging post not merely for commercial vessels but for British Antarctic expeditions). In this sense, the British presence in the Falkland Islands is beyond reproach. The problem is to reconcile this with the apparently implacable conviction of the government of Argentina that the Falkland Islands belong to Argentina.

Sovereignty is a heady concept, for which people are all too willing to kill each other. In the weeks ahead, the governments of Britain and of Argentina will also know that their own survival may depend on how things turn out in the Falkland Islands. So should they not think of trying to reach a settlement of this wasteful conflict by more old-fashioned methods than the use of military technology. Specifically, should not Britain think of offering to sell the Falkland Islands to Argentina in much the way in which Alaska was traded by the Soviet Union to the United States? The price, of course, would be high. Not merely would the 1,800 people of the Falkland Islands have to be compensated, but the British government would have to be recompensed for the loss of the benefits it now enjoys from possession of this out-of the way place as well as for the benefits that may in the future accrue.

Rewiring Britain

The Government, after a decade of indifference, is now too quickly embracing cable television.

As Paul on the road to Damascus, the British government has suddenly been converted to the belief that it will be socially beneficial if a substantial part of Britain can be covered with cable television systems. Two weeks ago (see Nature 25 March, p.282) the Department of Industry surprised most British taxpayers with an announcement that by the end of the year, it will have worked out a scheme that will allow commercial companies to invest in cable television systems. Between now and then, the government hopes, a committee under Sir John Hunt, one of the many exsecretaries of the Cabinet still in active service, will have worked out a set of rules for regulating this business, new for Britain. No doubt the government has it in mind that many voters at the next general election will go more cheerfully to the polls if they are able to return to a wider choice of television signals than at present.

The directness of the government's change of heart is remarkable. For the past decade, the Home Office has allowed only a few local experiments with cable television. Part of its reluctance is explained by its wish not to encourage competition with the development of the national broadcast television service. Thus, in the autumn and after years of argument, a fourth channel of broadcast television will take the air financed by the revenues from commercial advertising. Hitherto, the government has listened to the pleas of the Independent Broadcasting Authority that the success of that enterprise should not be jeopardized by allowing advertisers competing outlets for their cash. That principle, it seems, has now been abandoned. At the same time, the present British government has plainly decided that it can no longer allow itself to be restrained by the squeamishness of the principal opposition party about paytelevision. Subscribers to the proposed cable television networks will be able to watch on their screens whatever they can afford to pay for.

But what? This is where Sir John Hunt's committee will run into trouble. Working against the government's clock, the committee will be tempted to follow precedents established overseas, in the United States especially. It will thus be tempted to insist that any new cable system should distribute the four national television channels that are at present adequately served by conventional broadcasting. Even though some of the would-be operators of cable systems are likely to protest that such a requirement would be pointless, in the long run substantial benefits should accrue. The other obvious temptation will be to follow the common pattern in the United States which requires cable systems to provide one or more channels free to those local interests believing they have something to say to the public at large. That is a temptation to be resisted, for the experience of the United States has shown that people linked with cable systems vote with their tuning knobs to watch something else in preference. In the long run, the public interest will be best served by taking its chance with other interests.

The most contentious issue, however, will be that of how the content of signals put out on the new cable systems should be regulated, and by whom. There will be a temptation to set up some public body to ensure, for example, that nothing broadcast by the new cable systems can give offence to those who at present act as custodians of public morality. This, too, is a temptation that should be resisted. For the most obvious difference between cable systems and conventional broadcast channels is that the channel capacity of cable is much greater than that of the atmosphere. While it will remain important that the national television service should conform with rules that are understood and accepted, people linked with cable television should be allowed to receive whatever signals they wish — and which they are prepared to pay for. The danger here is that the potential operators of cable networks, anxious as they are to encourage the government in its new resolve, will meekly agree to censorship that will not afterwards be easily removed.

German cancer centre criticized

Report urges major changes in constitution

Sweeping changes at the Deutsches Krebsforschungzentrum (DKFZ) in Heidelberg have been recommended by the international commission appointed by the German Minister for Science and Technology. The proposed changes would invest the director of DKFZ with executive power, the lack of which caused the resignation of Professor Hans Neurath last year (see Nature 293,252; 1981).

The report, published last week by the ministry, is highly critical of the overall performance of DKFZ in basic research. Although acclaiming the quality of research by some investigators in the centre's eight institutes, particularly those of cell biology, immunology and virology, the report says that in a large number of the institute's 39 divisions, the research work is merely reliable and unimaginative. And the output of a number of scientists has amounted to little more than one unremarkable paper a year for the past decade: Overall "this does not indicate that the quality of research commissioned in DKFZ is in any way outstanding",

The centre is also criticized for its lack of collaborative ventures and its failure to take a lead in arranging multi-centre clinical trials. The commission considers that the largest cancer centre in Western Europe, employing more than 1,000 staff at an annual cost of DM90 million (£20 million), should do better.

Two main factors are said to account for the mediocre performance of DKFZ. The first is lack of effective external peer review. The second is that the constitution of the centre is such that the director does not necessarily have the power to bring about changes. "His proposals may be blocked or intolerably delayed and his position can be undermined by other proposals . . . from the Scientific Council" (a committee made up of the heads of institutes and an equal number of elected staff members). It was intolerable difficulties of this kind that Professor Neurath had "bravely attempted to overcome", says the report.

The resolution of this problem, says the commission, is of paramount importance to the future of DKFZ. It proposes that DKFZ's director (officially chairman of the executive board) should also serve as chairman of the Science Council. In this way the director could no longer be subordinate to his staff and could assume authoritative direction of the centre.

In the commission's view, the Science Council would still be a powerful advisory group whose majority view its chairman, the director, could ignore only at his peril (his is the only senior post without tenure).

On peer review, the commission says there is no adequate system to ensure that resources are wisely distributed among a staff most of whom have unlimited tenure. Some form of peer review has periodically been carried out by the centre's Science Advisory Council (Wissenschaftliche Beirat) but the council is not required by the constitution to conduct such reviews, is too small to perform the job adequately and is open to influence from the staff.

The commission proposes that the Science Advisory Council be replaced by a committee of eight independent scientists who would be responsible for arranging regular external peer reviews of all institutes and divisions of DKFZ. The same independent scientists would also fill the eight places for scientists on the Kuratorium. No longer would three of these posts be filled by elected members of

DKFZ. The commission considers that staff members have no place on the executive board of the institute that employs them whereas the director, who is not a voting member of the Kuratorium, should be on the board.

It is on the question of "worker participation" that there is bound to be political opposition to change. Staff representation on the Kuratorium began in 1975, when it was being widely adopted in both public and private concerns throughout Germany. Dr Wolfgang Finke, now chairman of the Kuratorium, would prefer to see it retained on the grounds that the presence of staff scientists on the Kuratorium has been a useful channel of information between the staff and the trustees. If there is a problem, he feels, it arises from the individuals concerned, not the principle.

Apart from that point, Dr Finke believes that the Kuratorium should give serious consideration to all of the commission's

Yale says no to a \$33,000 grant

Washington

Yale University last week reluctantly bit one of the hands that feed it, and declined a \$33,000 grant offered by the National Science Foundation for one year beginning on 1 May. By this unprecedented act, the university also gave renewed publicity to the fact that one of its faculty, mathematics professor Dr Serge Lang, strongly objects to the US government's requirement that recipients of federal research grants should produce detailed reports of the amount of time they spend working on federally supported projects.

Dr Lang described himself on the telephone last week as one "who knows more about A21 than anybody else". The reference is to the much disputed circular A21, issued by the Bureau of the Budget (now the Office of Management and Budget, OMB) more than twenty years ago. Yale's refusal of the grant, the renewal for a third year of its support for Dr Lang's project "Number theory and elliptic curves", is due to his refusal to accept the reporting requirements.

Dr Lang also said last week that his objections to "effort reporting" were unlikely to be met by the revision of A21 now being considered by OMB, which are substantially those agreed last year between OMB and groups such as the American Association of Universities and the Council of Scientific Society Presidents.

Since the publication of the proposed revision in the *Federal Register* on 7 January, there has been a steady flood of protests from academic scientists that even the proposed revisions do not meet the objection that it is meaningless to attempt

to distinguish between time spent on teaching and time spent on research. As published, the revision of A21 would do away with the requirement for a detailed account of the time spent on various activities by faculty members supported partly by federal funds. Instead, three alternative methods of accounting for effort devoted to federal projects are proposed, one of which would allow of a certifying signature by "a person having direct knowledge of the work" and not by the faculty member as such.

In the draft revision, however, OMB has not conceded the principle urged by many academic scientists that there are circumstances, research by graduate students for example, in which it is literally meaningless to seek to distinguish between research and teaching. Opponents of OMB's accounting ambitions are able with relish to quote on this point from the report of a task force in 1968 under Cecil E. Goode, then an official of the Bureau of the Budget.

Part of the reason why A21 has become a source of contention in the past three years is that government auditors have been seeking to use it as a means of quantifying (and presumably limiting) the indirect costs added to research grants as compensation for the administrative work carried out centrally by a university. Yale was not impelled to decline the first two instalments of Dr Lang's three-year grant because of a dispensation which expired at the beginning of this year.

Under the terms of the grant offered, Yale would have been paid two-ninths of Dr Lang's salary by way of compensation for work done during the long vacation.

recommendations. He will no longer be its chairman when the Kuratorium comes to consider the future of DKFZ on 21 June because, from 1 May responsibility for the centre will be shifted as part of a reshuffle of responsibilities in the ministry. It is likely that the new chairman will be Dr Güentsch.

Professor Hans Neurath, now back in the University of Washington, Seattle, also welcomes the report, describing it as "excellent, clear and succinct".

Reaction to the report at DKFZ is hard to gauge, but inevitably mixed.

Only after the 21 June meeting of the Kuratorium will it be known which of the commission's proposals will be adopted. One problem is that since Professor Neurath's resignation, DKFZ has been without a real director, although Professor Otto Westphal was appointed acting director from 1 March until the end of 1982. Another is that there is almost certain to be strong resistance to many of the proposals from within the centre. And it does not help that the politicization of the centre's difficulties comes at a time when the German government has matters of far greater importance on its plate - survival for example.

Thus, whereas the commission recommends that proper peer review of DKFZ starts without delay, with at least three institutes to be reviewed by the end of the year, there is a good chance that the peers will need topcoats rather than safari suits by the time they descend upon Heidelberg.

Peter Newmark

Europe's nuclear power

Border incidents

Brussels

A bizarre mixture of Molotov cocktails and red and yellow balloons was released at a demonstration on 27 March when Belgian environmentalists protested at the continuing construction of four French nuclear power stations at Chooz, close to the Belgian border. Protests against the Chooz reactors are now a regular part of Belgian life. Some weeks ago, the Belgian Embassy in Paris was occupied by two Belgian senators belonging to the environmental party, and demonstrations on the border are now promised on the last Saturday of each month.

While there is no sign that the protests will influence the French government's determination to press ahead with the four 1,300 MW reactors to be built on the Meuse at Chooz, they may affect the Belgian government's willingness to participate in the project. Given the prospect that Belgium will have to rely on nuclear power for half of its electricity production by 1985, the government must either sanction the nuclear power construction in Belgian or join in the Chooz project.

For a time, the government sought to obtain electricity and to counter the protests by linking participation at Chooz

with the stipulation that the French reactors should be built to Belgian safety standards. This suggestion has, however, been rejected by the French on the grounds that construction costs would be increased.

The issue has thus been invested with national pride. The Belgian government is being told, most vociferously by the Flemish-speaking population near the border, that it has allowed itself to be bullied by its larger neighbour. France, on the other hand, cannot admit that its own safety standards for reactors are inadequate simply because the Belgian standards are more stringent. France has also declined to reveal details of the reactors to be built at Chooz beginning in December, and to reveal plans for the discharge of some low-level waste into the Meuse.

There is a chance that the European Commission may be able to help. The revision of Article 37 of the Euratom Treaty soon to be adopted would require France to seek approval for its plans for radioactive waste disposal six months before construction at Chooz begins. So far, however, there is no sign that France is prepared to let the commission interfere or to engage in consultations with Belgium.

The Belgian government is in any case itself divided over the country's energy plans. The energy minister, Mr Etienne Knoops, is anxious that Belgium should have more nuclear power stations and keen on participation at Chooz. He is opposed by the science and budget minister, Mr Philip Maystadt, who wants the decision on Chooz put off until the parliamentary debate arranged for the end of May — after the 16 April deadline for a decision on participation will have passed.

In the end, Maystadt's arguments are likely to prevail. Belgian utility companies argue that it would be more sensible to invest in a Belgian project than in Chooz, especially because it is now estimated that Belgium will need to build thirteen 1,300 MW reactors by the year 2020. In any case, the cost of electricity from the Chooz plant quoted by the French is unpalatably high.

Jasper Becker

•Environmentalists are also on the move in France — on foot, in fact, between the site of the near-complete French fast breeder reactor, Superphénix, in the south of France, to Paris, where they hope to meet President Mitterrand on 18 April to protest against fast breeder development. A hundred demonstrators gathered on Sunday near Superphénix in the march, which has begun peacefully. This is in marked contrast to the rocket attack on Superphénix last summer, and the violent demonstrations of 1977 in which one demonstrator died. Brice Lalond, national secretary of the French Friends of the Earth, addressed the marchers. "Nothing has changed since 10 May", he said. referring to the election of Mitterrand as President of France and his socialist Robert Walgate government.

United States astronomy

High priorities

Apparently unabashed by the austerity now rife in Washington, the long-awaited Field report on astronomy and astrophysics in the 1980s will include a \$1,900 million (at 1980 prices) shopping-list, mostly for new equipment. A draft of the report (to be published next month) says that these plans would require that the astronomy budget of the National Science Foundation (NSF) in the 1980s should be 30 per cent greater in real terms than in the 1970s.

The report has been prepared by the Astronomy Survey Committee under the chairmanship of the director of the Harvard-Smithsonian Center for Astrophysics, George Field. The committee was set up in 1978 by the National Academy of Sciences (NAS) to assign priorities to a wide range of astronomical projects based on the ground or in Earth orbit, but excluding planetary missions and deep-space probes.

Field report recommendations Decade cost (1980 \$ million) Major new programmes, in order of priority 1. Advanced X-ray Astronomy 500 Facility (AXAF) 50 Very Long Base-line (VLB) Array 100 3. New Technology Telescope (NTT) 4. Large Deployable Reflector in Space 300 950 Modest new programmes, in rough order of priority 1. Augmentation of Explorer satellite programme 150 Far UV spectrograph in space 60 Space VLB antenna Optical/IR telescopes (2.5 m) 20 Advanced Solar Observatory in space 200 100 Cosmic ray experiments Search for Extraterrestrial Intelligence 20 750 Small new programmes 4 10 m submillimetre-wave radio antenna Other important programmes Spatial interferometer for mid-IR 3 High precision optical astrometry 3 programme Temporary programme to maintain astronomical expertise at 10 universities 20 Total expenditure over decade on new project 1,720 Total expenditure on "prerequisites 190 (theory, data analysis, etc.) Grand total \$1,910 million

In its review of astronomical problems and the best ways of tackling them, the report assumes the existence of such shuttle-dependent projects as the Space Telescope, the Gamma-Ray Observatory, the Shuttle Infrared Telescope Facility, the Solar Optical Telescope and the ground-based 25-metre millimetre-wave radiotelescope, all but the last of which appear to have survived in the US Administration's budget for 1983.

For the 1980s, the committee recommends that the highest priorities should be given to the Advanced X-ray Astrophysics Facility (AXAF) satellite, a Very Long Baseline (VLB) array of radiotelescopes, a

giant New Technology Telescope (NTT) in the 15-metre class and a 10-metre sub-millimetre-wave antenna. All these devices would contribute to the solution of the astronomical problems highlighted in the report — the large-scale structure of the Universe, the evolution of galaxies, violent events (such as supernovae and processes in active galaxies and quasars), the formation of stars and planets and solar and stellar activity.

In the committee's view, AXAF is a must at a cost of \$500 million. The VLB has beaten the 15-metre telescope into second place because it would require considerably less preliminary development and would have by far the best angular resolution of all techniques - 0.3 milliarc seconds possibly allowing the resolution of the nuclei of active galaxies and quasars at radio wavelengths. But because of the tremendous potential light-gathering power of the 15-metre telescope, an order of magnitude larger than present telescopes, the committee puts that on as high a level of scientific desirability as any other project examined.

The committee also urges increased support for what it calls prerequisites — instrument design (including a request for declassification of certain infrared detectors), theory, data analysis, computing facilities and laboratory astrophysics. To handle the data from AXAF, the committee recommends that a special institute be set up in support of the satellite, analogous with the Space Telescope Institute. As a consequence of its recommendations, the committee sees the need for an increase in personnel at all levels.

In his foreword to the report, Herbert Friedman, chairman of the Mathematical and Physical Sciences Assembly of NAS, stresses the need for an increase in the relative contribution of ground-based astronomy and, consequently, the NSF contribution. He also says that the technology of new developments in astronomy is dependent on but will also contribute to areas of science and to industrial and military applications.

In the present climate, it is hard to see the astronomers having many major desires fulfilled. Of the major recommendations of the influential Greenstein report, the 1972 predecessor of the Field report, only one - the VLA radiotelescope - was implemented in the less austere 1970s, for example. The Field committee emphasizes, however, that the level of expenditure it requests is equivalent in real terms to that which followed the Greenstein report. Moreover, Dr Friedman points out that they are hoping "that the purposes of the present fiscal policies will be achieved in a reasonably short period and that a healthier base of federal scientific support will then be restored". But if the Greenstein report can be viewed as a precedent, X-ray astronomers have struck lucky. Philip Campbell

High-energy physics

Tunnel vision

A legal obstacle to the construction of LEP, a European particle accelerator which should put European physics in the 1990s well ahead of the United States, has been removed. But the shouting is not yet

LEP - a large electron positron collider - will straddle the French-Swiss border in the plain of Geneva, linking with existing machines at the European Centre for Nuclear Physics, CERN. CERN has won cash and approval for the project from its member states, which include France and Switzerland, but the problem lately has been how to carry the project through local planning procedures in France and Switzerland. French environmentalists had managed by a legal technicality to block construction of an important exploration tunnel, but now the French court of appeal, the Conseil d'Etat, has finally ruled that the tunnel can go ahead after all.

This, however, is only a minor victory. The main justification for the tunnel was that it would provide an escape route for both engineers and water if the main LEP ring — which originally led right under the 1,000 m high peak of the Jura mountains — encountered high pressure water in the Jura limestone. But now the LEP ring has been shuffled almost entirely out of the Jura, by tipping it on its side (see figure), so water pressures will be lower. It will also now be possible to intervene from the surface if people are trapped.

However, there are still geological and political problems to solve. Geologically, the 3 km of LEP that will still lie in the Jura limestone lie entirely in the *piémont*, near the boundary with the sandstone of the plain. This rock is likely to be traversed by caverns and faults, and although vertical borings along the new route have revealed nothing serious, uncertainty persists.

Politically, the obstacle may still be the Association gessienne de protection de la Nature, the local French environmental organization presided over by M. Jean-Roger Honorat, the mayor of Echenevex. This group blocked the construction of the exploration tunnel, and although CERN has wooed it ever since, it remains suspicious.

The group will play an active part in the French planning process now under way.

New cancer research award

The 1982 Bristol-Myers Award for Distinguished Achievement in Cancer Research has been awarded to Dr Denis Burkitt (of Burkitt's lymphoma) and Dr Michael Epstein (of the Epstein-Barr virus). Their \$50,000 award was presented to them in New York this week.

Dr Burkitt was Government Surgeon in Uganda some twenty years ago when he proposed, from epidemiological studies, that an infectious agent caused childhood lymphoma in Africa, Dr Epstein heard Burkitt speak in London





Epstein (left) and Burkitt

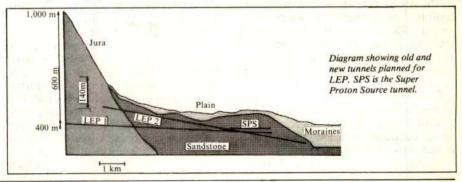
in 1961 and within two years had isolated the infectious virus that causes the lymphoma.

Burkitt is now an honorary senior research fellow at St Thomas's Hospital in London. Epstein is professor of pathology at the University of Bristol. And Yvonne Barr, in case you were wondering, is an Australian housewife.

Peter Newmark

The French ministry of foreign affairs, with which CERN must deal, now has CERN's final environmental impact statement, and under French planning law will act on CERN's behalf. There is to be a local public inquiry at which anyone in the region may present his views. CERN itself will be represented at the inquiry only if needed to explain technicalities; it has no right to plead. Eventually the results of the inquiry and files of submissions will be returned to Paris for a decision by the Conseil d'Etat.

Meanwhile, CERN is about to invite tenders for the construction of the tunnel, which should be in by the autumn. A decision from the Conseil d'Etat is not expected before the end of this year but a delay beyond that would mean LEP would not be producing particle collisions by the end of 1987 as planned. Robert Walgate



Agricultural Research Council

Savings plans

The British Agricultural Research Council (ARC) is still in a fix about its budget for the next three years. Plans to find all the necessary economies to meet the expected shortfall in its government grant at the Animal Breeding Research Organization (ABRO) and the Long Ashton Research Station have been abandoned. A decision on the future of the two institutes, which was to have been taken at the end of last month, has been put off to the end of April, and the council has set up a "core group" to look for comparable savings elsewhere.

ARC had originally planned to save the expected £3 million deficit in its annual budget by cutting research on food, beverages and fruit at Long Ashton and by cutting back ABRO to fundamental animal genetics. It has, however, found its hands tied by the Rothschild customer-contractor principle, whereby the Ministry of Agriculture (the customer) commissions a substantial proportion of research in ARC institutes (the contractors). The ministry, aided by the livestock industry, seems to have objected strongly to the plan to cut ABRO to one-fifth of its present strength. Such is the colour of the ministry's money, however, that at one stage it had almost persuaded ARC to cut fundamental rather than applied research. In the event, ARC last week produced a revised plan that may get it off that hook.

The revised plan being circulated for consultation before a final decision on 20 April entails a 50 per cent cut in ABRO's annual budget, from £2.25 million to £1.2 million, by 1984–85, but keeps the balance between fundamental and applied research roughly the same as now. Work on the long-term breeding of sheep and pigs and on the efficiency of dairy cows would continue, as would several areas of animal genetics. ARC says that it may also find a further £0.4 million for research in molecular biology later on. The council clearly hopes its new plan will have an easy passage.

The proposals for the Long Ashton Research Station, which have caused less of a stir, are likely to stand. Hence some research will probably be moved to the East Malling Research Station and the fruit industry is being asked to increase its support for the remaining programmes.

The debate over the future of the two institutes nevertheless has wider implications. ARC's own staff has complained that the process of regularly reviewing research programmes is not sufficiently open. It is also clear that the council needs some way of deciding priorities within as well as between institutes. But the most telling lesson of the past few days is the council's discovery that under the customer—contractor principle, the customer is ultimately (if not always) right.

Judy Redfearn

Not enough toxicologists in Europe?

The European Science Foundation (ESF) and the European Medical Research Council (EMRC) have announced a new programme for research and training in toxicology. A number of awards will be made in 1983 along the lines of ESF's existing brain research programme.

EMRC is basically a talking shop for ESF, representing members of the governing bodies of 14 Western European medical research councils who meet twice a year, and directors of the regional office of the World Health Organization and of the National Institute of Health in Bethesda, Maryland have a standing invitation to attend meetings. Toxicology has been a major interest of EMRC since it was established in 1971, and now nine members of EMRC have put up the money for the toxicology programme; the 1982 budget is FF700,000 (£63,600).

Although the demand for toxicologists in industry and government is growing rapidly, toxicology remains an ill-defined subject which may mean different things in France and, say, Denmark.

EMRC has recommended that priority should be given to mechanisms of toxicity and the epidemiology of exposure to chemicals. It also emphasizes the need for more research in toxicology and the inclusion of toxicology in the university curriculum. In the United Kingdom, for example, toxicologists are drawn mainly from pathology and biochemistry, and the subcommittee report on which the ESF programme is based is impressed with the complaint from industry that university graduates lack practical training in toxicology.

As well as travel and subsistence grants for training courses, ESF plans to award one-year training fellowships to graduates from toxicology-related disciplines. There will also be renewable fellowships for people already working as toxicologists for projects involving collaboration with teams abroad.

ESF is optimistic about the programme. It is to be hoped that the length of time it has taken to set up the new programme is not indicative of the pace at which all collaborative European research will develop.

Jane Wynn

Soviet science and technology

Practical slant

The Soviet Academy of Sciences seems to be planning a more direct interest in production research, according to Dr Anatolii Aleksandrov, the president, in his address to the academy's annual meeting. The academy's increased participation in technological projects in the past eighteen months seems to be connected with the appointment of Dr Gurii Marchuk, formerly chairman of the Siberian branch of the academy, as head of the State Committee for Science and Technology.

When Dr Marchuk moved to the state committee, which is responsible for applied research and its implementation, he urged greater use of the academy's talents in implementing research results in production — thus bridging what has become a major planning gap.

The academy's new practical slant is clearly approved by Dr Aleksandrov, who has often stressed that the Soviet Union is potentially independent of foreign research and development. By contrast, the chief academic secretary of the academy. Dr Georgii Skryabin, told the annual meeting of his regret that the deterioration of the international situation has led to reduced contact with the West. He particularly deplored the cutback in exchanges with the United States - although the US National Academy of Sciences had asked for talks to continue on international security and arms control. Contacts with the United Kingdom, on the other hand, had, he said, "become more active".

University research funding

Line of defence

Washington

US universities and colleges continue to benefit from Washington's current enthusiasm for increasing its expenditure on military technologies and related national security needs. A subcommittee of the House of Representatives has now approved a provisional bill, said to have wide support within the Administration, which would allocate up to \$100 million a year to improve facilities in higher education institutions in technical subjects important for security interests.

The explicit purpose of the legislation is to increase the ability of the United States to meet both its immediate defence needs and the technological demands that would be created by a prolonged war.

The legislation is supported by the Department of Defense, the Department

"HERE'S YOUR GRANT—THIRTY THOUSAND PIECES OF SILVER."



of Commerce and congressmen representing areas of the United States which are suffering hardest from the current recession.

Furthermore, according to a report published last month by the Defense Science Board (DSB) on the state of the nation's research universities and their ability to absorb increased military research funds, many universities which had rejected close involvement with the Pentagon in the late 1960s and early 1970s are now experiencing a change of heart.

The DSB study has been used by the Department of Defense to justify the major increase in university research funding which it is asking Congress to support. In his budget request last month, President Reagan proposed a 30.5 per cent increase in university basic research funded by the Army, to a total of \$92.1 million; a 36.9 per cent increase in such research funded by the Air Force, to reach \$89.7 million; and a 17.2 per cent increase in Navy funding. Basic research supported by the Defense Advanced Research Projects Agency would rise to \$8.4 million, a 21.7 per cent increase. Many of the extra funds would go explicitly to raise the level of effort in the mathematical sciences, electronics, chemistry and engineering conversion.

The details of the Defense Department's budget request reflect some of the science board's conclusions and some of the universities' concern. In response to complaints about the declining state of the universities' research facilities, the department has announced its intention to allocate \$150 million over a five-year period to improving research instrumentation. Each of the three major services will put aside \$10 million a year for this purpose.

The department is also attempting to overcome the increasing difficulty of universities in retaining top graduates in fields such as engineering by setting up a new graduate fellowship programme. Stipends will be \$12,000 a year — about 50 per cent higher than comparable awards made by the National Science Foundation — and the services will, in addition, provide affiliated universities with \$8,000 a year to cover tuition and fees.

The initial plans are to award about 125 graduate fellowships in the fiscal year 1983. which starts on 1 October 1982, costing the Department of Defense about \$2.5 million. Dr Jack Crowley of the Association of American Universities has said that the association warmly supports both the proposed funding for research facilities and the new fellowship programme.

If Congress does grant the extra funds there is unlikely to be any shortage of applications for the additional grants. With support for basic research from other agencies by falling in real terms and with industry unlikely to be able to make up much of the shortfall, the universities are looking to the Department of Defense as the only significant alternative source of support.

David Dickson

Oceanographic research

In the black

At a time when many groups are hard pressed to support large projects, a team from the UK Institute of Oceanographic Sciences (IOS) has just returned from a successful six-month cruise on which it earned more than £250,000 in foreign currency. IOS, now run by the Natural Environment Research Council, has been able to share the cost of its unique side-scan sonar project GLORIA (Geological Long-Range Inclined ASDIC) by combining its interests with those of several research groups abroad. With the ability to scan a swathe of the ocean floor almost ten times wider than conventional deep-tow sonars, the equipment is quicker and cheaper than other survey techniques, and many groups overseas are apparently eager to use it.

Research on this multi-project charter—
the first long voyage to use GLORIA—
was strongly oriented towards commercial
applications, principally oil exploration
and radioactive waste disposal. The UK
Department of Energy commissioned the
first part of the voyage, with an extensive
survey off Newfoundland aimed at
completing a reconstruction of the North
Atlantic during the Cretaceous to help



GLORIA aboard the trawler Farnella

assess the oil potential of Britain's continental shelf. The other British project, from the Department of the Environment, was an investigation of the possible disposal of high-level radioactive waste under the sea bed. Searching for specific sites is not the primary objective at present; the department is more interested in using GLORIA's ability to distinguish fine textural changes in the ocean floor to study the varying types of sea-bed environment.

One of the most exciting finds came from an area known as the Amazon Cone off the Brazilian coast. This is a huge pile of sediments washed out by the Amazon into the Atlantic, and is thought to be a potentially important source of oil. Submarine current meanders were for the first time observed to cut into the cone, giving new clues about processes of sediment deposition that may be relevant to the formation of oil-bearing structures. There has been great interest in this finding from petroleum geologists and from the Brazilian oil company Petrobras, which paid for this leg of the cruise jointly with

the Lamont-Doherty Geological Observatory of New York's Columbia University.

During the last leg of the voyage, on the way home from Miami, GLORIA was tested as a means of detecting deposits of manganese nodules, which at present can be located only by trial and error sampling. There seems to be a chance that GLORIA's sensitivity to slight variations in the reflectivity and roughness of the bed may offer a more direct means of location. United States scientists are said to have been impressed with the equipment.

David Millar

Soviet shake up

A regional earthquake monitoring centre has been opened in Dushanbe, in the Soviet republic of Tadjikstan, as a first step towards a seismic early warning system for the whole Soviet Union. Tadjikstan suffers several thousand earth tremors a year, and seismic activity is also very high in the neighbouring republics of Turkmenia, Uzbekkstan, Kirghizia and Kazakhstan.

The new centre will combine the resources of existing seismological centres of the Academies of Sciences of the various republics in the area, including a seismic testing ground covering 30,000 square kilometres in the northern Tien-Shan. The facilities of this site include wells 3,000 kilometres deep for studying subterranean waters in particular the radon content of such waters which has been observed to increase shortly before a tremor. Field stations at the site also monitor the Earth's magnetic field and telluric currents, while microdisplacements of individual crustal blocks are measured with lasers. Biological teams monitor the behaviour of snakes, ants and other fauna said to behave abnormally during the build-up to an earthquake.

In 1980-81 Soviet seismologists say they successfully predicted the occurrence of 13 earthquakes, including force 4-5 tremors in Dushanbe. The seismologists seem very open-minded to any suggestions of possible early-warning signals. Recent investigations have included the relationship of the amount of mercury vapour emitted from the Earth's crust, or present in subterranean well water, and an assessment of local folk lore which has it that a seismic shock is frequently preceded by overcast skies and dust-storms.

The heart of the Dushanbe centre will be a computerized seismological data bank, linking existing centres, to be augmented by links to new mobile seismic monitoring stations scheduled to go into production in the near future.

Vera Rich

CORRESPONDENCE

The return of malaria to India

SIR - In their article on malaria resurgence, Chapin and Wasserstrom1 claim that the agronomic use of DDT led to the development of DDT resistance in mosquitoes and hence to a resurgence of malaria in India. However, the main cause of the rapid increase in the consumption of insecticides in India since 1970 has been their greater public health use in the wake of malaria resurgence2, and agricultural use of DDT has remained fairly constant during the past several years (see table). So the correlation between increase in DDT use and rice production (Fig. 1 of Chapin and Wasserstrom) is superficial and does not imply a cause-effect relationship. Similarly, noting static cotton production and increasing total DDT consumption, Chapin and Wasserstrom are incorrect to conclude that "more and more DDT must be sprayed simply to maintain a fixed vield'

The public health use of DDT in India has always exceeded its agronomic use, so although the agricultural use of insecticides has contributed to the resistance problem, blame must be shared by both agricultural and public health applications. And moreover, as multiple resistance in Indian mosquitoes to DDT, BHC and malathion is not cross-extending³, correlation between the use of a single insecticide (DDT) and malaria cases (Fig. 2 of Chapin and Wasserstrom) can only be spurious.

When seeking reasons for the present problems for malaria control in India administrative lapses are a factor which must be considered. Under the World Health Organization (WHO), moves in the 1950s to reduce Plasmodium transmission by attacking female mosquitoes via insecticide spraying, and to deplete the Plasmodium reservoir in human beings by treating patients with antimalarial drugs, were successful. However, this success was nullified because mosquito populations in the forests of Assam, Madhya Pradesh and Orissa were not taken into account. These populations were not affected by spraying of dwellings, so remained infective and gradually reintroduced Plasmodium to the now resistant mosquito population. Here it should be noted that Chapin and Wasserstrom use "resistant mosquito populations" and "malaria" as synonyms, when in fact resistant mosquito populations existed harmlessly in India for a decade becoming a hazard only in the presence of Plasmodium.

In attempting to find ways of overcoming India's malaria resurgence, an example to learn from is that of California, where use of physical, cultural and biological techniques as adjuncts to chemical control has both prevented malaria resurgence and reduced insecticide use to about a third of the levels used a decade ago⁴. Such techniques have substantially cut the costs of mosquito control in California, and if they were adopted in developing countries, savings could be used to replace DDT with the alternatives which have not been used before because of the high costs.

Although what appears clear with hindsight may in fact have been difficult to foresee, it seems fair to say that WHO was slow to recognize the limitations of residual

Insecticide consumption in India, 1973-1979				
Insecticide	1973-74	1975-76	1977-78	1978-79
	Pul	blic health		
BHC	5.0	6.7	12.0	17.0
DDT	7.0	7.2	6.0	10.2
Malathion	0.5	1.0	1.1	5.5
	Ag	riculture		
BHC	15.4	18.6	16.6	20.0
DDT	2.9	2.9	2.5	3.0
Malathion	0.4	0.6	1.0	1.5
Carbaryl	2.5	3.5	2.0	3.0

Values are in millions of kilogrammes. The figures for 1978-79 are for projected consumption.

insecticides. WHO placed prime reliance on chemical control and gave low priority to nonchemical control measures so that when malaria control began to deteriorate, no proven alternatives were available to supplement insecticides. A review of literature on mosquito pest management strategies5 reveals that WHO's efforts in this line started in the 1970s, by which time mosquito resistance to insecticides had become rampant. Though it is difficult to differentiate between the residues from insecticide use in agriculture and public health, preliminary studies6 indicate that DDT and BHC use in malaria control may be responsible for the serious pollution by these components in malarious developing countries7-9. This side effect of malaria control with persistent insecticides has also received limited recognition from WHO.

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Helical liposomes

SIR — Lin et al.¹ have recently described the "induction of helical liposomes by Ca²⁺-mediated intermembrane binding", and the two types of helical liposomes discovered by Lin et al. were displayed on the cover of

Nature of 11 March. The authors acknowledged the independent observation of tubular and helical liposomes by another contemporary scientist. But it may be of further interest to your readers that two types of helical liposomes were in fact described some 78 years ago² (see figure).

The book by Lehmann² and a more recent

The book by Lehmann² and a more recent review³ may serve as guides to the extensive studies made after 1854⁴ on tubular and

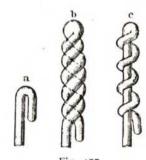


Fig. 477.

helical myelin figures (zopfartige Flechtwerke). Lehmann² also offers a mechanism for the formation of the two types of helical liposomes.

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French freedom

SIR — With reference to your important issue, Science in France (25 March), may I highlight one crucial advantage that French scientists enjoy over their British counterparts?

Applicants for a research grant, which may be rejected, have the statutory right of access, under France's freedom of information laws, to the case-file concerning their application, including referees' reports and other attendant reasons behind the decision, which they may then openly contest and indeed reverse¹.

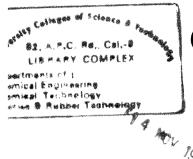
British scientists who feel that they order this matter better in France, compared, that is, with the closed and secretive system of administration in Britain, would do well to make strong representations to their MPs and trade unions ^{2,3}.

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NEWS AND VIEWS



Geochemical light on mantle convection

from Donald L. Turcotte

Mantle convection is generally accepted to be the driving mechanism for continental drift and plate tectonics, but controversy persists about the scale on which the process takes place. Does convection involve the whole mantle, down to the boundary with the core? Or, as Allègre¹ has recently suggested on geochemical grounds, are only the upper layers of the mantle involved? Is there even, as Houseman and McKenzie² suggest, a second scale of mantle convection?

The controversy centres on the seismic discontinuity at a depth of 650 km, where seismic velocities increase by about 10 per cent. The increase can be attributed either to a solid-solid phase change or to a change of composition, perhaps of iron content. The absence of seismicity below this depth is taken as evidence that the convection associated with plate tectonics is limited to the upper mantle.

Geochemical observations lend further support to the notion of layered mantle convection. Systematic studies³⁻⁵ of isotopic ratios in the samariumneodymium system have shown that the upper mantle and the continental crust are complementary geochemical reservoirs. The upper mantle reservoir is systematically depleted in those elements, the rare earths for example, which are incompatible with the mantle material and the continental crust is correspondingly enriched. The mass balance of the Sm-Nd system suggests that 30± 10 per cent of the upper mantle has been involved, which is consistent with an upper mantle convection system limited to depths of less than 650 km.

A possible reservoir system is illustrated in the figure. Mid-ocean ridges, which randomly tap the upper mantle reservoir, usually yield mid-ocean ridge basalts which are similar in both major and trace element chemistry, showing that the upper mantle reservoir is well stirred. Volcanism associated with the subduction zones systematically transfers incompatible elements into the continental crust. The remainder of the subducted lithosphere, including the enriched oceanic crust and depleted mantle, must then be mixed into the upper mantle reservoir in order to provide the nearby uniform and depleted basalts found at mid-ocean ridges.

This simple two-reservoir model does not, however, explain all the geochemical observations. Ocean island basalts (for example, Hawaii) are not systematically depleted but exhibit a range of geochemical signatures, from depleted to enriched. One explanation is that ocean island basalts result from partial melting in a plume rising from an undepleted lower mantle reservoir6, in which case the range of compositions is attributed to mixing between materials of the depleted upper mantle and the undepleted lower mantle. This model does not, however, explain all enrichments of ocean island basalts. whence the proposal⁷⁻⁹ that the source of the ocean island basalts is subducted oceanic crust, enriched with continental trace elements by seawater alteration and by the entrainment of sea-floor sediments10, that has not been fully mixed into the mantle.

In passing, the two-reservoir model must

also be amended to take into account the discordance between geochemical studies of the Sm-Nd system on the one hand and the strontium-rubidium and uranium-lead systems on the other. Fractionation within the crust seems to have depleted its upper layers of lead and strontium, suggesting a differentiation of the crustal reservoir into two. Whether the 'missing' lead is in the lower crustal reservoir or (as sulphide) in the molten core is disputed.

While these geochemical studies favour a layered mantle convection system, there are some difficulties. Thus Jordan and Creager¹¹ have suggested that travel time anomalies indicate the penetration of the descending surface plate to depths of at least 900 km, which argues against stratified convection.

If indeed the mantle is chemically stratified at 650 km or thereabouts, independent convection systems would be expected in the upper and lower mantle. If the observed discontinuity marks a phase change, its thermal coupling between the two systems will depend on the thermodynamic properties of the phase change. High-pressure measurements 12 using diamond cells with laser heating indicate that the principal upper mantle mineral olivine transforms to basic oxides (magnesiowustite plus perovskite) at conditions reasonably close to those at a

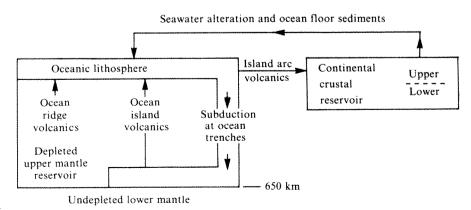


Illustration of the mantle reservoirs.

Donald L. Turcotte is Chairman of the Department of Geological Sciences at Cornell University, Ithaca, New York 14853.

depth of 650 km, but not enough is known about this transformation to decide whether it would assist or block convection. Moreover, Liu¹³ argues that there is a significant discrepancy between predicted and required conditions for the olivine transformation, and favours chemical stratification.

Further objections have been suggested by the laboratory and numerical studies of stratified convection carried out by Richter and McKenzie¹⁴, who predict a double thermal boundary layer at the interface between the convecting layers with a significant increase of temperature across it. But this increase of temperature would imply¹⁵ a large (several orders of magnitude) decrease in viscosity which is not consistent with observation of postglacial rebound. This conclusion is a serious difficulty for the concept of stratified convection which is unlikely to be recovered by a consideration of dependence of the viscosity or rheological differences between the convecting layers.

That there may be a second and smaller scale of mantle convection is suggested by several observations. One is intraplate volcanism, such as that at Hawaii and other oceanic islands. Another is the requirement that the upper mantle reservoir be well stirred or mixed in order to have a nearly uniform composition. Richter and Parsons¹⁶ suggested that instabilities within the lithosphere may be responsible, but Yuen, Peltier and Schubert¹⁷ have questioned the validity of the numerical

studies so far made of this process² on the grounds that they do not allow for the strong temperature dependence of the mantle viscosity. They go on to suggest that the hot, low-viscosity lower boundary layers are more likely to be unstable than the lithosphere. And, such unstable lower boundary layers could help them to explain the ocean island volcanism.

It is, of course, too soon to known how these questions will be decided. What is, however, already plain is that geochemical methods will throw light on the mechanisms of mantle convection, which are not merely the driving forces of continental drift but powerful agents in the evolution of the planet.

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Parental mutagenesis and familial cancer

from H.J. Evans

PEOPLE can inherit mutations which predispose their offspring to the early development of specific cancers, and which are loosely referred to as 'cancer genes'1; and mutagen-induced chromosomal rearrangements, or more subtle mutations, may initiate malignant transformation in exposed somatic cells². Does this mean that exposure to mutagenic carcinogens results not only in an increased incidence of cancer in exposed individuals, but also in their descendants?

It is recognized that abdominal exposure of pregnant women to X-rays may result in an increased incidence of leukaemia in the resultant offspring⁴; that maternal exposure to synthetic oestrogens during pregnancy can result in the subsequent development of vaginal adenocarcinoma in female children⁵; and that exposure of children to asbestos dust brought home from the work place by an occupationally exposed parent is responsible for the later developmental of mesothelioma in exposed offspring⁶. More recently, an excess of

brain tumours has been noted in children of adults occupationally exposed to chemical solvents⁷. But in all these studies, the excess cancers are the result of the exposure of the offspring themselves and are not a consequence of gonadal exposure of parents and transmission of a cancerpredisposing genotype to the offspring. Indeed, the evidence in man for transmission of any kind of induced germ cell mutations to offspring by parents exposed to chemical mutagens is almost nonexistent, with some limited evidence from studies of individuals exposed to vinyl chloride8 or to anaesthetic gases9,10. There is no information available on any possible germ cell transmission of an acquired cancer risk from parent to offspring. What evidence do we have from experimental

H.J. Evans is Director of the MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XJ. studies on animals?

Following the original report of transplacental carcinogenesis in pregnant female mice exposed to urethane¹¹, there have been a number of studies on tumour incidence in offspring of pregnant animals exposed to chemical mutagenic carcinogens. In some cases, inbred lines of mice12 or rats13 have been used and brother-sister matings followed through to the F₂ or F₃ generations. All reports show that treatments that result in tumours in exposed individuals and their immediate descendants also result in an excess of tumours in F₂ and F₃ animals. That the enhanced tumour incidence in later generations is not due to transmission of the carcinogen through mother's milk or excreta was ruled out through the use of rapidly metabolised and inactivated mutagens such as N-nitrosomethylurea (NMU)¹³ or N-nitrosoethylurea (ENU)¹⁴. Moreover, in a recent study, Tomatis et al. 15 have shown a similar increased incidence of tumours in F₁ offspring of male rats treated with ENU some weeks before mating, so that a mutagen-induced enhancement of tumour incidence can also be transmitted through the male germ line. The story is taken further by the large-scale study reported by Nomura in this week's Nature in which tumour incidence in offspring of male and female mice exposed to X rays or urethane has been followed over a number of generations.

On page 575, Nomura summarizes a study extending over ten years, and involving some 15,000 mice, and reports that exposure of late-stage male or female germ cells to X rays gives a linear increase in dominant lethals with dose, but no increase when animals are exposed to the mutagen urethane; however, parental exposure to both agents resulted in congenital abnormalities in F₁ embryos. Of special interest is the finding that both X-irradiated and urethane-treated male or female mice produced progeny with a much increased tumour incidence, with a clear doseresponse relationship for offspring derived from irradiated late-stage germ cells. At the highest X-ray dose of 500 rads, the tumour incidence in the offspring of irradiated animals (~30 per cent) was some six times that in the controls (5 per cent). The spectrum of tumours in controls and in offspring of treated parents was similar, with some 90 per cent being non-malignant papillary adenomas of the lung and the remainder various malignant tumours of other organs. Matings continued down to the F₃ generation clearly demonstrated heritability of the induced high tumour incidence and the pattern of inheritance is that of a dominant trait with 40 per cent penetrance. Although the X-irradiation of males resulted in dominant lethals and cytologically demonstrable translocations, no such effects were observed in animals treated with urethane, and Nomura argues that the mutations that result in excess tumours are probably not gross

chromosomal rearrangements.

The rate of X-ray induction of mutations that predispose to tumour development turns out to be around ten times greater than the mutations that, for example, result in skeletal changes in the mouse and Nomura suggests that there may be a large number of sites in the genome where mutation may predispose to tumour development. Other explanations are possible, but whatever mechanism is involved it is clear that mutagen exposure results in transmitted heritable changes that considerably enhance tumour incidence in the offspring. The demonstration of this effect required parental exposure to fairly high doses of mutagens and the use of inbred lines and we may ask whether these results are of significance in relation to human exposure to mutagens. There is no reason to believe that effects similar to those in the mouse may not occur in man, although they would be less easily demonstrated. In this context, two human populations of interest would be the offspring of the survivors of Hiroshima and Nagasaki who were exposed to high radiation doses, and who showed an enhanced cancer incidence and chromosome damage in their blood cells 16, and the offspring of cigarette smokers who must comprise one of the largest groups of individuals exposed to a chemical mutagen at dose levels sufficient to produce significant chromosome damage in their blood lymphocytes 17.

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With increasing molecular size, however, new problems arise. First, there is the inability to resolve the resonances; increasing molecular weight increases not only the number of resonances but also their peak width. With hundreds or even thousands of proton resonances spread over a frequency range of only a few kilohertz, considerable overlap is inevitable. Even with resonances that can be resolved, an even greater problem is presented by their assignment. Not all the factors affecting chemical shift can be analysed exactly, so that a peak at a given frequency can only be definitely assigned to its corresponding nucleus by direct experiment. This often means chemical modification of the molecules, or comparison of almost-identical proteins, and the labour required for even one or two positive assignments in the spectrum of a protein can be enormous. Once assigned, however, the chemical shift, splitting and other features of the resonance can provide sufficient information about its local chemical environment, including dynamic behaviour, to make the effort well worthwhile.

The work presented by Wuthrich et al. represents the culmination of several years of development, in their laboratory and others (notably that of Ernst), of new NMR techniques which not only provide solutions to the problems of resolution and assignment, but further point to a scheme for the determination of protein structure in three dimensions directly from NMR experiments. The methods are those of two-dimensional NMR, in which the sample is subjected to a sequence of radiofrequency pulses of the general form 'pulse-evolution time t_1 - pulse - data acquisition time t2'. The data are thus collected as a function of t_2 with t_1 as a parameter, and if a large number of experiments are perforned for different values of t_1 the result is a matrix of data points, each of which is a function of both t_1 and t_2 . This matrix is Fourier-

New dimensions in protein NMR

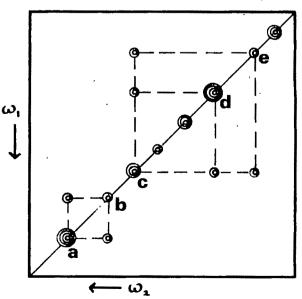
from H. W. E. Rattle

NUCLEAR magnetic resonance (NMR) has been much in the news of late; the obvious biochemical and medical applications of such techniques as topical magnetic resonance (TMR) and NMR scanning have aroused a great deal of interest. Arguably, though, an even greater revolution has been going on in the application of NMR to the determination of the molecular structure of proteins, and a recent sequence of papers14 from Wüthrich and his colleagues shows clearly that it may soon be possible to derive structural information from peptides and the smaller proteins in solution at the same levels of resolution as we have become accustomed to seeing from X-ray crystallography.

When the first protein samples were put into 40 and 60 MHz NMR spectrometers in the mid 1960s, they produced a spectrum of three or four broad featureless humps which made it clear that much work would be needed before useful information could be obtained. The advance of high-field magnet technology produced great improvements, as did the rapid data acquisition made possible by the Fourier transform method. The nuclear spins of the sample are perturbed by the application

of a radio-frequency pulse, and their subsequent collective behaviour monitored as a function of time. Fourier transformation of the resulting amplitude—time function produces the NMR spectrum with each resonant nucleus in the molecule giving rise to a peak at a position on a frequency scale (the chemical shift) which depends on its precise environment within the molecule.

Schematic representation of a two-dimensional COSY or NOESY contour-map spectrum. The peaks of the conventional proton magnetic resonance spectrum appear along the diagonal, and crosspeaks reveal the connectivity between resonances a and b, and between c, d and e. (Adapted from ref.5.)



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transformed twice to yield a twodimensional spectrum in which peaks appear as functions of two frequencies ω_1 and ω_2 . In the presentation favoured by Wüthrich et al., the spectrum is plotted as a square contour map with the peaks of the conventional spectrum appearing along a diagonal. Clearly more data can be presented in two dimensions than in one. but the real advantage goes far beyond this. By the selection of pulse sequences and appropriate values for the evolution time, each pair of nuclei which interacts within the molecule is made to reveal its 'connectivity' by the appearance of an offdiagonal peak (a cross-peak) on the spectrum. The connectivities may be through-bond spin-spin couplings (J-couplings), in which case the technique is termed two dimensional-correlated spectroscopy (COSY), or across-space connectivities produced at ranges of up to a few Angstrom units by the nuclear Overhauser effect (NOESY) (see the figure). Since nuclear Overhauser effects vary with distance, it should eventually be possible to measure short internuclear distances between nuclei not directly linked by bonds; for which at present only approximate values are obtainable.

The general scheme for using COSY and NOESY proton spectra is then as follows: spectra are obtained from a peptide of known sequence in aqueous (H2O) solution, and at least one backbone a-CH or N-H resonance assigned by conventional means. Spin coupling of this resonance to those of adjacent residues along the chain produces cross-peaks, thus permitting assignment of peaks from the neighbouring residues. These in turn have cross-peaks with the next residues, and so the whole protein backbone may be assigned sequentially. Across-space NOE connectivities obtained from the NOESY spectrum add to and confirm the assignments; detailed analysis of known protein structures show that between 70 and 95 per cent of close approaches are between contiguous residues. Virtually complete assignments of every proton in the molecules of basic pancreatic trypsin inhibitor (58 residues) and membranebound glucagon (29 residues) are presented as proof of the efficacy of the method. Given such full spectral assignments, an even more interesting prospect appears, since NOESY spectra can give at least semiquantitative values for the distances between the α -CH proton of the *i*th residue and the backbone NH proton of the (i+1)th, between backbone NH protons of adjacent residues, and between B-CH or β -CH₂ of residue *i* and NH of residue (i+1). The conformation of a residue is as completely specified by these distances as it is by the more conventional Ramachandran bond angles ψ , ϕ and χ . Given enough NOESY measurements of such distances, even if only approximate, and using the other known constraints on the folding of the protein, it should be possible

with appropriate computer methods to arrive at a fairly complete determination of protein conformations in non-crystalline environments, at least for proteins smaller than 70-100 residues. Of course COSY, NOESY and other two-dimensional NMR methods may also be used in conjunction with the many valuable conventional methods, such as paramagnetic probes, relaxation measurements, biochemical modifications and others which are already in regular use. The instrumental requirements for two-dimensional NMR are considerable: high spectrometer

frequencies (300-500 MHz) coupled with computing facilities capable of handling a two-dimensional spectrum which may be the equivalent of several hundred conventional spectra. The advances made possible, however, are very considerable.

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The big bang — 'free lunch' at the Royal Society

from Craig Hogan

COSMOLOGISTS seem to have spent a great deal of time over the years talking about the same things over and over again: element abundances, background radiation, galaxy formation and the like. In so doing they tend to oscillate between congratulating themselves on how much they think they understand and despairing that they will never know anything for certain. Both of these moods were much in evidence at a discussion meeting held recently at the Royal Society, supposedly on "The Big Bang and Element Creation" but actually covering a wide range of subjects intended to span the whole range of observational cosmology and a good deal of theoretical cosmology.

As expected, the most genuinely new results came from groups measuring anisotropy in the microwave background radiation. D.T. Wilkinson (University of Sussex), whose experiment is capable of measuring large-scale (90°) angular fluctuations in radiation temperature with a system noise of only 0.1 mK (compared with the total 3K background temperature), described a possible observation of a cosmological quadrupole moment with amplitude $\delta T/T \simeq 10^{-4}$ which, if true, would be an observation of historic significance, because such measurements observe structural properties of the Universe at very early times, possibly even the big bang itself. Unfortunately, as Wilkinson noted, such observations are beset with numerous systematic difficulties expected to be only slightly fainter than the observed signal, and this became obvious when he showed a sky map made with a new low-noise maser detector in which the Galaxy appeared as a bright band that was much more conspicuous than any trend in background brightness. R. Fabbri (University of Florence) described results from the

Craig Hogan is in the Institute of Astronomy, University of Cambridge, Madingley Road, Cambridge CB3 0HA. Florence group and cited tentative evidence that cosmological fluctuations at 6° angular scale are smaller than the quadrupole ($\delta T/T = 3 \times 10^{-5}$) and decreasing with angle — a result as exciting as Wilkinson's, but difficult to reconcile with it in any simple picture. On the other hand, Wilkinson showed very clearly that the dipole moment ('the great cosine in the sky' due to the Earth's motion) is now firmly established, even if minor differences still exist between various groups.

In the same tone, P.L. Richards (University of California, Berkeley) showed that the spectrum of the background radiation is indeed very nearly blackbody down to submillimetre wavelengths, even though controversial apparent departures from an exact thermal spectrum may exceed the statistical limits. Woody and Richards' technological achievement may be appreciated by noting that the cosmological background they measure had a brightness only 10-5 of an ordinary body at room temperature. New experiments were described which may check the significance of the spectral distortion, which again, if real, gives us significant positive information about the early evolution of the Universe.

Measurements of the cosmic abundance of the elements - to date, the only independent verification that the microwave background is primordial were reviewed by B. Pagel (Royal Greenwich Observatory). Progress has been made here too, with the best measurements (optical observations of HII regions) of primordial helium abundance by mass giving $Y_p \sim 0.23 \pm 0.01$, a conservative statement being $Y_p < 0.25$. As pointed out by D. Schramm (Enrico-Fermi Institute, University of Chicago), with $Y_{\rm p} = 0.25$ the hot big bang predicts the observed abundances for deuterium and ³He with remarkable success.

However, the helium observations now

also place serious constraints on the standard hot big bang model. M. Davis (Center for Astrophysics, Cambridge, Massachusetts) showed evidence from the flow of galaxies in the Virgo supercluster that the total mass density of the Universe is at least one-fifth of the density which would be necessary to keep it from expanding forever in terms of the conventional cosmological density parameter, $\Omega_{TOTAL} >> 0.2$. Most of this mass is dark material whose nature is unknown. If, however, it were made of ordinary matter, the hot big bang predicts that the amount of helium produced would exceed the observed limits. If the standard model of helium synthesis is correct, then $Y_{\rm p}$ < 0.25 implies that the fraction of closure density in baryons is $\Omega_{\rm B}$ < 0.1. Therefore, the standard model already requires that most of the mass in the Universe is some other form of matter for example, massive neutrinos, or black holes which formed before nucleosynthesis, or something even more exotic. As Schramm pointed out, the standard model would be ruled out altogether if Y_p proved to be less than Pagel's 'best' value of 0.23. In view of this sensitivity, the success of the theory must be regarded as even more remarkable.

W. Sargent (Caltech) pointed out that deuterium may appear in high-resolution spectra of very distant objects — the Lyman- α absorption due to hydrogen clouds in front of high-redshift quasars. Such systems provide direct, if fragmentary, information about the intergalactic gas at redshift of order 2, and have already been used by Sargent and his collaborators as a probe of galaxy formation processes.

The simplest prediction of all was made by A. Guth (MIT), who showed that in some circumstances, a tiny region (or 'bubble') of primeval vacuum could inflate to include the entire observable universe, then decay to fill it with all of the matter and radiation.

The reason such a scheme is possible in the context of modern gauge theories is that the primordial vacuum is allowed to have a large energy density. When such a 'false' vacuum expands, its energy density does not decrease (like ordinary matter) but stays the same, which enables the universe to expand by a very large factor while remaining at the same density. The universe becomes 'normal' when the energy of the vacuum is converted into ordinary matter and radiation (by a decay process which is not well understood, and which is the focus of research), and the vacuum becomes the normal, nongravitating 'true' vacuum we have today. (We know that the energy density of the present-day vacuum is nearly zero because the 'cosmological constant', which measures its effect on the present-day expansion, is shown by observations of distant galaxies to be nearly zero.)

Such an 'inflationary universe' auto-

matically predicts that $\Omega_{TOTAL} = 1 \pm \epsilon$ (where $\varepsilon << 1$), and, because of the uniformity of the vacuum, also predicts that no primordial fluctuations are produced — the early Universe is both very flat and very uniform. It appears that in this kind of scheme, one will have to find an explanation for the observed nonuniformity in terms of processes occurring after the inflation. However, the most interesting of Guth's speculations is that the bubble of vacuum, and hence the entire universe, could arise by quantum fluctuation 'from nothing'. Apparently certain kinds of gauge symmetry might predict not only the structure of matter but also the structure and existence of the space time in which the matter fields operate.

Nowadays, not only does modern physics give you electrons and atoms, but it also gives you universes. Guth's vision of the Universe as a 'free lunch' will appeal to many. I walked away afterwards (as it turned out, to go to lunch) feeling perplexed as if by a koan — if the Universe owes its existence and symmetry to gauge theory, does the gauge theory owe its 'realization', and hence its existence or 'reality', to the Universe it creates? No doubt these speculations will provide cosmologists with plenty of food for thought.

When is a zeolite not a zeolite?

from Lovat V.C. Rees

In 1972 the Mobil Oil Corporation synthesized and patented a range of very high Si/Al zeolites of which ZSM-5 is the best known example. This zeolite is a catalyst of great importance in the synthesis of petrol from coal (see Thomas et al., this issue of Nature p.530) and the Mobil patent is thus of considerable commercial significance. As Thomas et al. indicate, the Union Carbide Corporation has more recently synthesized and patented a new, thermally stable, crystalline phase of silica, called silicalite, which is topologically very similar, if not identical, to ZSM-5.

Silicalite, however, always contains some aluminium (Si/Al ratios >200 are usually found) which Union Carbide assert is present in the form of Al₂O₃ impurity. The Mobil patent for ZSM-5 embraces Si/Al ratios of up to 4,000, so the zeolite may contain less aluminium than some samples of the silicalite. If some of the aluminium in silicalite is present as tetrahedral framework aluminium, then a philosophical argument arises as to whether a sample of ZSM-5 (US Patent 3702886, 1972) with very low aluminium content is in any way different from silicalite (US Patent 4061724, 1977). This philosophical argument, which could have considerable financial implications, can be carried a stage further when one asks "When is a zeolite not a zeolite but a defect silica"?

If the aluminium content decreases below one aluminium atom per unit cell, can we still consider the material to be an aluminosilicate with a specific unit cell formula or is it a defective form of crystalline silica? Even if one aluminium per unit cell is present, if this aluminium atom is randomly distributed among all tetrahedral sites in the unit cell is this material

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The paper from Thomas and his colleagues at Cambridge and Guelph goes to the heart of this question. They have shown by ²⁷ Al magic-angle spinning NMR (MASNMR) that all of the aluminium atoms in a very 'pure' silicalite (Si/Al>1,000) and the less 'pure' commercial silicalite (Si/Al>200) are located in tetrahedral sites. No noctahedral aluminium was detected. They also found a small signal in their 29Si MASNMR spectrum of the commercial silicalite due to Si(1Al) which must be present if the aluminium is present in tetrahedral coordination. If these results are accepted then we must draw the conclusion that silicalite is essentially indistinguishable from a high Si/Al ratio ZSM-5 and leave the question of whether these materials are

Zeolites are crystalline aluminosilicates which are widely distributed in nature but are also readily synthesized in the laboratory. The general formula for a zeolite may be written as:

 $M_{x/n} [(A1 O_2)_x (Si O_2)_y] mH_2O$

where M is a cation of valency n and the Si/Al ratio can range from one to very large values. In many zeolites, the zeolitic water can be readily removed without lattice collapse, leaving a porous crystal with void volumes which can be as large as 50 per cent of the crystal volume. This void volume consists of a network (frequently three-dimensional and interconnected) of channels of molecular dimensions. Zeolites have found wide use, therefore, as molecular sieves and as acid catalysts. When the M cations are replaced by H+ ions, very active cracking, hydrocracking and isomerization catalysts are produced which are extensively used by the petroleum industry.

zeolites or not unanswered.

Another novel application of 29Si and ²⁷Al MASNMR, and some further exciting results, are presented in this issue in a second, joint contribution from the above two schools (see p.533). Zeolite Y (synthetic faujasite) as synthesized is an excellent cracking catalyst but is found to be inadequate under plant use when high temperatures are employed in the decoking. process. The conversion of zeolite Y to an ultra-stable form was an important development which has led to the production of >200,000 tons of ultrastable zeolites per annum. Although the

main features of this stabilization process have been indicated in previous publications, the new work presents convincing evidence of the fate of the aluminium atoms removed from tetrahedral framework sites and of the healing of the vacancies so produced by silicon atoms which come, not exclusively from surface or amorphous regions of the crystal, but also from the bulk crystal. A direct probe has been brought to bear, therefore, to monitor the internal reorganization of tetrahedral atoms which converts a catalyst into a commercially viable catalyst.

What next will we find by MASNMR? \Box

Much of the best evidence comes from platelets, where receptor-coupled disappearance of PI almost certainly involves the activity of a phosphodiesterase (a phospholipase C), whilst Ca2+-mediated PI disappearance is caused by an acylhydrolase (a phospholipase A)9,10. Only the former is of any immediate interest when considering the possible coupling function of receptor-controlled breakdown of inositol lipids. Neither the experiments of Farese¹¹ on pancreas nor of Cockcroft¹² on leukocytes demonstrate the route of Ca2+-dependent PI 'breakdown' in receptor-stimulated cells.

Hawthorne correctly points out that the data on bovine adrenal medulla are not easily reconciled with the inositol lipid breakdown/Ca²⁺ mobilization hypothesis. Additional information on this system should be illuminating.

It has generally been considered that inositol lipid metabolism is stimulated by activation of muscarinic cholinergic receptors at both pre- and postsynaptic sites, and Hawthorne states this view as a fact. Recent work, however, has shown that in 'synaptosomal' fractions at least a proportion of this response is associated with postsynaptic elements¹³, and I¹⁴ have offered additional arguments to suggest that there may be no presynaptic stimulation of PI metabolism by muscarinic cholinergic stimuli. If this were to prove the case, then the failure of presynaptic muscarinic receptors to mobilize Ca2+ would tally well with their failure to stimulate inositol lipid metabolism.

Hawthorne states that "there are so many systems in which the receptor-linked PI loss requires external Ca2+ that a universal role of PI in Ca2+ mobilization cannot be upheld". This statement is fundamentally flawed, since it fails to recognize that a requirement for extracellular Ca2+ does not constitute proof that a change in cytosol Ca2+ concentration controls PI breakdown (see ref. 8 for a detailed discussion).

For the past few years it has been generally held that the first lipid to break down in stimulated cells is PI rather than one of its phosphorylated derivatives,

Is phosphatidylinositol really out of the calcium gate?

from Robert H. Michell

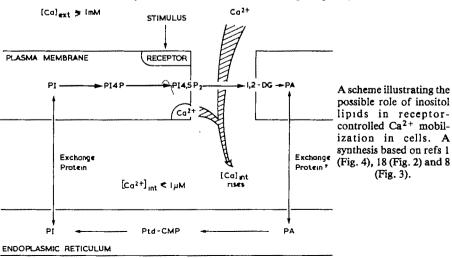
Seven years ago I suggested that receptorstimulated breakdown of phosphatidylinositol (PI) might be implicated in a general mechanism whereby activation of many cell-surface receptors brings about a rise in the cytosolic Ca2+ concentration in stimulated cells1. In recent months, two brief reviews, one by J.N. Hawthorne in these columns, have argued that this general hypothesis is no longer tenable^{2,3}. .By contrast, a number of other recent reviewers have continued to treat it as a useful working hypothesis, despite emphasizing that it is still unproved4-8. I would like briefly to summarize some of the weaknesses of Hawthorne's recent News and Views article3. I feel that these criticisms are sufficiently fundamental to raise serious doubts about the purported disproof of the postulated link between stimulated inositol lipid breakdown and the mobilization of Ca2+ in stimulated cells.

Hawthorne considers that the proposed mechanism for Ca2+ mobilization is cumbersome, mainly because not all the necessary enzymes are at a single cellular site, and that it is wasteful of energy. But membrane lipids are always in a state of continuous metabolic turnover, and the rapid synthesis to support this continuous turnover occurs mainly at the endoplasmic reticulum. The inositol lipids of the plasma membrane that are involved in cell responsiveness may simply be renewed by intracellular synthesis rather more rapidly than the other phospholipids of the plasma membrane. When considering how much energy this costs, a comparison with adenylate cyclase may be instructive. Synthesis of cyclic AMP expends two highenergy phosphate bonds (plus an unquantified consumption of GTP by the guanyl nucleotide-binding coupling protein) and yields a single molecule of the intracellular

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messenger. Breakdown and resynthesis of one molecule of PI consumés three highenergy phosphates (assuming conservation and reutilization of the diacylglycerol mojety). If the intiating event turns out to be phosphodiesterase attack on PI 4.5-bisphosphate (PI4,5P₂; see below), then this expenditure will rise to five highenergy phosphate bonds. As a result of this breakdown, we can assume the release of some membrane-bound Ca2+ and/or the opening of a Ca² channel. Thus the breakdown of one lipid molecule might allow the mobilization of many Ca2+ ions into the cytosol compartment of the cell. The three (or five) phosphate bonds seem likely to be a minor energetic consideration, since most energy expenditure during Ca2+ signalling undoubtedly occurs in the maintenance of the large Ca2+ gradient at the plasma membrane by continuous pumping of Ca2+ out of the cells, rather than in the control of Ca2+ re-entry.

As Hawthorne mentions, there are situations in which the admission of Ca2+ to cells, either by receptor activation or by using an ionophore, can cause the disappearance of PI, but in no case has the route of breakdown been fully documented.



possible role of inositol lipids in receptorcontrolled Ca2+ mobilization in cells. A synthesis based on refs 1 (Fig. 4), 18 (Fig. 2) and 8 (Fig. 3).

PI4P and PI4,5P2. Breakdown of either one or both derivatives was only reported in conditions likely to raise cytosol Ca2+ concentrations greatly, and studies of the enzyme responsible for their Ca2+mediated breakdown in red cells indicated that this enzyme is very unlikely ever to be controlled by Ca2+ in healthy cells15. Recent studies of liver and parotid cells have, however, revealed a second type of PI4,5P, breakdown that is very rapid, closely coupled to receptor occupation and not Ca2+-mediated (refs 8, 16, 17 and unpublished data of L.M. Jones and C.P. Downes). This raises the novel possibility that the initiating reaction in Ca2+ mobilization is receptor-controlled breakdown of PI4,5P2, a characteristic lipid of the plasma membrane, and that the disappearance (that is, apparent 'breakdown') of PI so far studied is really a reflection of the consumption of PI during resynthesis of degraded PI4,5P₂. This does not constitute any fundamental change to the original view that the widely observed 'PI response' is somehow essential to Ca2+ mobilization by receptors, rather it acknowledges our increasing knowledge of the mechanism of this response: originally we had to discuss the function of stimulated PI and phosphatidate labelling, later we were able to focus on PI 'breakdown', and investigation of receptor-controlled PI4,5P, breakdown may now take us one step closer to a final understanding of this long-standing puzzle. The figure shows our current working hypothesis.

In no sense should the information that appears to link breakdown of PI4,5P₂ (or PI) to the mobilization of Ca²⁺ in stimulated cells be taken as conclusive proof of this link. We are still much too ignorant of the details of the processes to draw any such conclusion. Equally, however, I consider that the evidence marshalled as a purported disproof of this hypothesis is far less convincing than Hawthorne³ and Cockcroft² would have us believe. Let us not reject this working hypothesis prematurely.

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X-chromosome inactivation and the control of gene expression

from Martin H. Johnson

WHAT is the relationship between the state of chromatin and the expression of genes? The heterochromatic and inactive X-chromosome appears to offer an appealing model system to tackle this question, since during X-inactivation (or reactivation) the alteration of potential genetic activity, and its inheritance through many cell generations, involves simultaneous changes to a large and coherent group of genes rather than to a series of genes scattered throughout the genome. During the course of a recent meeting in Bangalore*, it became clear that this assumption was too simple. It emerged that the condensation of the X-chromosome, and indeed of heterochromatin elsewhere in the genome, may be a secondary phenomenon, perhaps related primarily to the evolution of sex, and that the selective activity of genes during differentiation might be regulated by other mechanisms altogether.

In the eutherian mammal, the embryo begins life with two active X-chromosomes, but the paternally derived one is later inactivated in trophectodermal and primary endodermal cells (N. Takagi, University of Hokkaido). The cells resemble those cell lineages in adult marsupials in which the paternal X-chromosome is inactive (it is partially active in some lineages), although it is not clear whether this is a result of failure to activate the paternal X-chromosome at fertilization, or of activation followed by preferential inactivation (J. Graves, LaTrobe University). An impressive array of cytogenetic, biochemical and embryological evidence suggests that, in the primary ectoderm (epiblast) of eutherian mammals, random rather than selective inactivation of the X-chromosome occurs between 51/2 and 61/2 days (Takagi; M. Monk, MRC Mammalian Development Unit, London; M. Lyon, MRC Radiobiology Unit, Harwell).

The simultaneous occurrence of X-inactivation, whether random or paternal and in embryo or embryonal carcinoma cell (Takagi), and the generation of differentiated cells from a stem cell population, lead Monk to propose that both arise from a single genetic change. She suggests that X-inactivation may be just one part of a more general process of genetic determination and that both events might be mediated by the same molecular mechanism. Experiments in which X-inactivation is reversed complicate this simple but attractive idea.

Three types of reversal were described. First, A. McLaren (MRC Mammalian Development Unit, London) presented evidence that the germ cells, although stem cells, had an inactive X-chromosome during their mitotic proliferation in the primitive mouse gonad of 111/2 days gestation. Biochemical, genetic and morphological evidence showed clearly that between 12½ and 13½ days both X-chromosomes became active at some point before entry into prophase of the first meiotic division. Second, T. Mohandas (University of California, Los Angeles) exposed mouse-human cell hybrids (Science 211, 393; 1981), in which the human locus for hypoxanthine phosphoribosyltransferase (hprt+) was on an inactive X-chromosome, to 5-azacytidine, an agent known to reduce DNA methylation (see below), and selected in HAT medium. Clones of hybrid cells, in which human HPRT activity was stably expressed for over six months in culture, were generated and extracted DNA was shown to transform HPRT-negative cells. Graves similarly found that 5-azacytidine treatment of an F, cell line derived from a hybrid Mus musculus × M. caroli (in HPRT-deficient clones of which the M. caroli X-chromosome was inactive) resulted in stable re-expression of the M. caroli form of HPRT. Loss of HPRT activity in thioguanine-resistant revertants accompanied loss of the M. caroli X-chromosome, confirming that the reactivated locus was still on the X-chromosome. In six per cent of reactivant clones, the M. caroli form of glucose 6-phosphate dehydrogenase (G6PD) was also expressed, suggesting that reactivation events at the two loci were not independent.

Finally, Graves described experiments in which the hprt+ locus on the inactive M. caroli X-chromosome was reactivated simply by cell hybridization, either with undifferentiated embryonal carcinoma stem cells or with male-derived mouse or Chinese hamster fibroblasts. Again, the loss of HPRT activity in segregants was concordant with loss of the M. caroli X-chromosome. The reactivation frequency induced by fusion was much lower than that induced by 5-azacytidine. Recently, de Jonge et al. (Nature 295, 624; 1982) have also reported reactivation of the inactive human hprt + gene independent of 5-azacytidine treatment; DNA from human cells carrying an inactive hprt+ locus was used to transform HPRT-

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^{*}The conference on "Condensed Chromatin and the Human X-Chromosome" was organized by H. Sharat Chandra at the Indian Institute of Science, Bangalore, India, 14-16 December 1981.

negative mouse fibroblasts and expression of the hprt+ gene was detected.

In experiments involving reactivation either by 5-azacytidine or by cell hybridization, the inactive X-chromosome remained heterochromatic and allocyclic. Although HPRT activity was being selected for, G6PD and phosphoglucokinase activities were also expressed, but only occasionally. A selective reactivation of discrete loci thus seems to have occurred — a process quite distinct from the natural reactivation described by McLaren in which the entire X-chromosome ceases to be heterochromatic and expression of any or all loci can occur. This selective reactivation is reminiscent of the continuing activity of X-linked loci Xg and Sts on otherwise inactive, allocyclic and heterochromatic X-chromosomes. The X-chromosomes may thus be inactivated at the level of individual genes (which can be switched on or off as can any developmental genes on autosomes) or at a higher level where the chance of individual genes switching on is reduced. The use of 5-azacytidine or cell fusion for reactivation may somehow selectively penetrate the higher-order suppression. That conclusion made it seem less likely that X-inactivation will prove to be a good model for studying the control of gene activity during development.

The capacity of 5-azacytidine to induce reactivation of X-linked loci is consistent with a model for X-inactivation first put forward in 1975. R. Holliday (National Institute for Medical Research, London) and A. Riggs (City of Hope Medical Center, Duarte, US) suggested that methylation of CpG sequences at replication would provide an inheritable change in the DNA that could be associated with suppression of activity and hence could also lead to X-inactivation. Suppression of methylation during one round of replication would generate hemimethylated strands which, on a second round of replication, would leave some cells carrying non-methylated sequences and other cells methylated sequences. Divergence of gene activity in different cell types could thus be achieved. Two rounds of DNA synthesis do indeed seem to be required between each determinative event during early mouse embryogenesis (M. Johnson, University of Cambridge).

Reactivation by 5-azacytidine may occur because it substitutes for cytidine but cannot itself be methylated. However, Jones and Taylor have observed that the extent of demethylation induced by 5-azacytidine is significantly greater than would be expected from substitution alone, and may thus involve a more generalized suppression of methylase activity. If demethylation by 5-azacytidine does occur on such a large scale, why is the whole X-chromosome not reactivated instead of just one or two loci? It seems clear that methylation changes are not in themselves adequate to explain the phenomena

observed — a conclusion supported by a recent report of Wolf and Migeon (Nature 295, 667; 1982). They compared the methylation patterns of DNA from cells with active or with active + inactive X-chromosomes by Southern blot analysis using cloned X-chromosome-specific probes and found no evidence to support any relationship between X-chromosome inactivity and degree or pattern of methylation.

Exciting news came from K. Jones (University of Edinburgh) who described a probe of approximately 200 base pairs, highly repeated, made to the minor density fraction of satellite DNA quantitatively associated with the W chromosomes of the banded krait, a snake in which the female is the heterogametic sex and the W chromosome is therefore equivalent to the Y chromosome of mammals. Remarkably, the Banded Krait Minor density fraction (BKM) probe also hybridizes to the W chromosomes of the females of a number of species of snakes, either at a single discrete area or at a series of sites along thechromosome; to bird W-chromosomes (female heterogametic); to regions tentatively indicated to be associated with the transposed and active mating-type locus (MAT) of yeast; and to the mouse (male heterogametic) Y-chromosome in the centromeric region. In addition, the probe bound to scattered sites elsewhere throughout the autosomes of mammals and also to mouse X-chromosomes carrying the 'Sxr gene'. The gene appears to be involved in primary sex determination as, when present in XX or XO mice, it causes a testis to be constructed. The origin of the Sxr locus has long been obscure, but Jones' demonstration that the highly conserved BKM target sequence is translocated from the Y-chromosome to the terminal arm of one X-chromatid during meiosis suggests that either the conserved

sequence, or more probably an associated flanking sequence, is the region coding for the sex-determining function of the Y-chromosome.

Jones suggests that this highly conserved sequence, in addition to its association with sex-determining loci, also defines functional domains in chromatin which are heterochromatic and allocyclic, as for example observed in the probe-positive region of the Y- and W-chromosomes, or is involved in the DNA conformational changes associated with conversion of yeast mating types from repressed to active states. The probe also hybridizes in the region of the proximal-most euchromatin (19A-D) and in the adjacent heterochromatin (19E-20B) of the Drosophila X-chromosome, a region proposed earlier in the meeting (D.L. Lindsley, University of California, San Diego) to be the site of sequences responsible for the precocious X-chromosome inactivation in the primary spermatocyte.

Jones proposed that the association of the highly conserved sequence with heterochromatic regions may have arisen in conjunction with the evolution of sex. If the sequence occurred on different primitive X-chromosomes to varying degrees, two classes of X-chromosome might develop. depending on the strength of the inactivating centres. The coincidence of strong centres on the chromosome carrying the putative sex-determining gene(s) could lead to genetic inactivity of the primitive X-chromosome at all times other than at the moment of determination of the male sex. Thus, most genes would rapidly be lost, generating a Y-(or W-) chromosome. With weak centres on the primitive X-chromosome lacking the sex-determining gene(s), activity would remain. On this basis, X-inactivation might be viewed simply as a relic of the requirement for a sex-determining chromosome.

100 years ago

THE ORIGIN OF THE SIGNS OF THE ZODIAC

THE origin of the signs of the zodiac is a question which we have but recently obtained materials for answering. Some of these symbols, certainly, are plain enough: it is not difficult, for instance, to discover the horns of the bull in the symbol of Taurus, or the arrow in that of Sagittarius. But the meaning of others, such as the symbols of Virgo, of Scorpio, or of Capricornus, is not so self evident. These symbols, however, are of comparatively modern invention, and first came into use along with the symbols still employed by astronomers to denote the planets. They cannot be traced further back than the tenth century, and owe their origin to the connection the alchemists believed to exist between the planets and the metals.

But modern though the symbols of the planets and zodiacal signs may be, it is quite otherwise with the signs themselves, and the majority of the names by which we still call them. Recent research has shown that the general voice of classical antiquity was right in regarding the Chaldeans as the first to map out the path of the sun during the year into separate regions, or constellations. Copies made by Assyrian scribes of older Babyloman works on astronomy have been found in the library of Nineveh, and are now in the British Museum.

It was the primitive population of Babylonia, now known by the name of Accadians, who first made Chaldea famous for its study of astronomy, and it is to them that the Signs of the Zodiac are due. Each sign represented a month of thirty days, and the signs and months were accordingly called by common names.

Some names clearly originated in the pecularities of the season when the sun was in a special sign of the zodiac. This is certainly the case with Aquarius, and it is probable that fish were particularly abundant under Pisces when the lowlands of Babylonia had been inundated by the rains.

From Nature 25, 525, 6 April 1882.

SPRING BOOKS SUPPLEMENT

Where there's a need for some style

In science-writing it is hard to combine accuracy with fluency. Here Eric Ashby calls for more trouble to be taken over literary style in government reports.

In St Matthew's Gospel it is written: "Thou shalt love thy neighbour as thyself". A more recent writer puts it another way: "It is desirable that there should be established a meaningful and responsive reciprocal personal relationship within the social group". Here the choice between plain prose and jargon is easy to make. But some messages cannot be coded into words anyone can understand. Every issue of Nature carries pages that are meaningless except to a coterie of experts. This is not because the articles are badly written. It would be unreasonable to expect the author of an article on "Sub-GeV antiprotons in galactic cosmic rays" to make his message intelligible to anyone who can read a newspaper. He can assume that the few score of fellow experts for whom he is writing regard the content as almost all that matters in the article. He must avoid inaccuracy and ambiguity; fluency and elegance in style, though welcome and likely to enlarge the circle of readers, are of secondary importance.

The science-writer who chooses a wider readership has a more difficult task. No one is obliged to read what he writes. His motive—to communicate or maybe just to make money—will be defeated if he doesn't take trouble to set out his ideas in lucid, even if he can't manage limpid, prose. He has to adopt a style acceptable to the market place. This exposes him to certain temptations.

The chief temptation is to strive to be lucid at the cost of being accurate. How bracing it is to breathe the fresh air of Macaulay's prose: and how mistaken to suppose that science can be written in that style. It has been unkindly said about Macaulay's style of writing that you can never tell the truth in it. "In satisfying his passion for clarity" wrote the philosopher Brand Blanshard, "he allows himself to omit shades and qualifications that are there in the facts, but would smudge his sharply etched lines if he were to put them into the picture".

The science-writer with a passion for clarity has to take care that his passion does not lead him into infidelity to truth; for the reader who at first takes pleasure in the "sharply etched lines" may in the end feel resentment at having been deceived. That is what befell the American authors of The Limits to Growth. They arrest the reader's attention with a vivid scenario, such as the one that pollution, if it gets worse, may almost halve the expectation of life in industrial societies. They take no account of the fact that industrial societies react strongly to any threat to the expectation of life - as a dozen laws to abate hazards to health bear witness and the arresting scenario is in fact deceitful. It is commonly said that without the "sharply etched lines", the reader would not have taken the trouble to read the book at all. This is the dilemma, and the fact that so few writers can resolve it (and so many do not even try) provokes scientists to frown on a colleague who goes in for popularization; the word is so uncomfortably close to the French vulgariser. The rare writer who can successfully combine accuracy with fluency is not frowned upon: he is envied. But for most of us who write about science it is safer to settle for being painstaking about accuracy and be content with a modest measure of fluency.

However there is one category of science-writing where there needs to be a campaign for more fluency, even for elegance, in style: that is in the reports of scientific committees set up by government. The purpose of these reports is to help to shape science-policy. The committees that draft them take immense trouble to make them trustworthy. But — with some outstanding

exceptions — little trouble seems to be taken to make them attractive to read. The consequence is that some reports are read only by those whose duty compels them. And no wonder: listen (it's better to read them aloud) to a couple of examples taken at random from two important reports. Here is a paragraph from the recommendations of the Finneston Report, *Engineering our Future*:

All those involved with manufacturing industry, whether directly or indirectly, should review their activities to ensure that they perceive and present engineering and engineers as matters of vital national concern in their own right.

Does this mean: "The importance of engineers for industry and for the nation needs to be stressed"?

And here is a passage from the recommendations of the report on Global Resources to the President of the United States:

Because of the risk to peace, the United States should encourage other countries, and should itself, establish conflict resolution arrangements which can mitigate or help resolve future international water conflicts.

Does this mean: "There is an urgent need for ways to resolve conflicts over competing demands for water"?

There is no need for reports to governments to be written in this stilted and stumbling style. There are models of elegance on both sides of the Atlantic. The report, A Race against Time, by the Royal Commission on Electric Power Planning in Ontario, and the report by Mr Justice Berger on the Mackenzie Valley Pipeline (Northern Frontier, Northern Homeland), make compelling reading. These are both from Canada, where there is a higher level of literacy in government reports than in the USA. In Britain we have the example of an experiment in fluent writing with, so to speak, a "control" issued in the same year by the same government department. In 1970 the Ministry of Housing and Local Government issued two reports, one from the Technical Committee on the Disposal of Solid Toxic Wastes and one from the Working Party on Sewage Disposal. Neither of these topics is likely to inspire felicity in style, but the contrast between the two reports is striking. That on solid toxic wastes is pedestrian, the format dull, the readership correspondingly small. The report on sewage disposal was a pioneer in the attractive packaging of government reports. The photographs on the cover, the clever diagrams, the homely style, all these say to the reader: "This is not written just for civil servants: it is for you".

Stimulated by pressure groups and the media, the man-in-thestreet is becoming more and more concerned about technical decisions which affect his life. If he cannot participate in the decision-making (in public inquiries and the like) he at least wants to understand on what evidence the decisions are made. It is therefore a prime duty laid on those who write the reports of advisory committees and commissions to make them attractive and lucid enough to appeal to a much wider circle of readers than a handful of politicians and civil servants. It would be a public service if reviewers of such reports were to pay attention to style as well as content, praising good writing when they find it and censuring prolixity. Whitehead wrote that it is more important that a proposition be interesting than that it be true. Reports from scientific committees to governments need to be both interesting and true.

Heavenly insemination

Hugh Montefiore

I SUPPOSE that it is no more strange that an outstanding biologist such as Francis Crick should give his views on cosmology than that a distinguished astronomer such as Fred Hoyle should pronounce on molecular biology. To an outsider, these are both welcome pointers towards an integration of the sciences. So perhaps it was not outrageously odd to give Crick's book to a theologian for review; but he must walk warily on alien territory.

Crick writes ostensibly to answer Enrico Fermi's famous question, "If there are

intelligent beings in the galaxy, why aren't they here?", and he assesses the hypothesis known as Directed Panspermia, that is to say, a variant of Arrhenius's nineteenth-century theory, modified in that Crick considers whether life was deliberately planted on Earth. His book has nothing in common with Hoyle's views propounded in Evolution from Space (Dent, 1981). Crick. for example, does not seriously question whether the period needed for life to emerge by natural process requires a time span longer than the age of the Earth. Nonetheless he seems somewhat sensitive to his wife's criticism that his work may seem more like science fiction than a Nobel Prize winner's critique of a scientific theory. Perhaps for this reason Crick takes the opportunity (as his title suggests) to give his view on life itself, its origin and nature. Written with enviable simplicity and free of abstruse technicalities, it is as a result devoid of any annotation.

The answer that Crick gives to Enrico Fermi is that life on Earth could well have originated elsewhere in the Galaxy, and that there has been time enough for intelligent beings to evolve elsewhere in a suitable environment and to have despatched prokaryotic and eukaryotic microorganisms by rocket to this planet, whence life here may have developed. Crick, however, admits that this theory of Directed Panspermia, although plausible, suffers from extreme paucity of evidence. He calls it "premature". Crick the human being affirms: "Once the scale and nature of the galaxy is appreciated, it is intolerable not to know whether we are its sole inhabitants"; but Crick the scientist is cautious: "I cannot myself see just how we shall ever

Life Itself: Its Origin and Nature. By Francis Crick. Pp.192. UK ISBN 0-356-07736-5; US ISBN 0-671-25562-2. (Macdonald/Simon and Schuster: 1982.) £7.95, \$13.95.

decide how life originated".

A small measure of support for Directed Panspermia is seen in the almost uniform nature of the genetic code. Some factors, however, Crick does not consider; for example, the origin of some bacteria with properties which seem unlikely to have



evolved to meet our earthly conditions. And it is surprising that he does not mention the microfossils which suggest that eukaryotes existed at least as early as prokaryotes, although the initial gap of a billion years means that we do not know how either evolved. As for the supposedly random emergence of life in the prebiotic broth, Crick writes:

It is impossible for us to decide whether the origin of life here was a very rare event or one almost certain to have occurred;

yet elsewhere he writes of it as "an infinitely rare event" and even admits that

an honest man, armed with the knowledge available to us now, could only state that, in some sense, the origin of life appears at the moment to be almost a miracle.

I wonder, however, whether Crick's view will continue to prevail that "it seems almost impossible to give any numerical value to the probability of what seems a rather unlikely sequence of events" leading to the emergence of life. In any case the really fundamental problems remain to be solved; how DNA, RNA and enzymes were originally formed, and how the first cell, with its reproductive mechanism, came into existence.

Whether all the dense and sober argumentation of Crick can be validated, I am not in a position to judge. Such matters as available environments for life to emerge elsewhere, the length of time involved, the mode of travel and the duration of the voyage cannot be more than scientific speculation. Crick's scientific writing is lucid, magisterial and suitably cautious ("seems to be", "apparently", "so far as we know"). But in Chapter 15 ("Why Should We Care?") the tone abruptly alters. Crick the Nobel Prize winner has given way to Crick the human being. He reminds me here of those days in Cambridge when he offered a cash prize for the best secular use of a college chapel. When it comes to religious beliefs, gone are the qualifications and dogmatic certainty takes over. "Most modern scientists do not subscribe to any of them" (has he carried out a scientific survey?); and

The plain fact is that the myths of yesterday which our forebears

regarded not as myths but as the living truth, have collapsed, and while we are uncertain whether we can successfully use any of the remaining fragments, they are too rickety to stand as an organized interlocking body of beliefs [italics mine].

Francis Crick's personal convictions will doubtless be read with respect; but he would put us in his debt if he would give some explanation of their logical connection with the natural sciences of which he is such a pre-eminent practitioner. Wherein, for example, lies a scientist's "almost boundless optimism concerning his ability to forge a new set of beliefs"? It might seem to some, that the dreadful threat of thermonuclear war overhanging

the civilized world, with one-fifth of all scientists at work on defence contracts. should fill us with almost boundless pessimism. Granted, however, "the tremendous success of science, especially in the last hundred years", what is the logical connection between scientific explanation and ultimate meaning, or between scientific knowledge and ultimate belief? Or again, what is the scientific basis for the phrase "outmoded religious beliefs"? For example, is a scientific evaluation of the origin of life compatible with a theistic concept of creation? Readers may wish that Crick had here argued his case rather than merely stated his conclusion. Seen from our anthropic viewpoint, a remarkable series of "coincidences" must have taken place onwards from the initial explosion which brought the cosmos into

being, so as to make possible the eventual emergence of Homo sapiens (whether or not ours is the only form of intelligent life in the Universe). These "coincidences" certainly occurred. They may be due to complex sequences of random and meaningless events. But they are also compatible with divine providence. Crick tells us that in considering the origin of life a "gut reaction" is likely to be superficial or misleading. Not all intuitions however deserve to be described in such pejorative terms. Spiritual insights are not to be despised as adequate explanations to such questions which, despite Dr Crick's almost boundless optimism, seem to transcend the fields of scientific investigation.

Hugh Montefiore is Bishop of Birmingham.

Wholes and parts, meaning and mechanism

Marie Jahoda

The Reenchantment of the World. By Morris Berman. Pp.353. Hbk ISBN 0-8014-1347-0; pbk ISBN 0-8014-9225-4. (Cornell University Press:1982.) Hbk \$43.10, £24.95; pbk \$11.10, £5.95. The Turning Point: Science, Society And The Rising Culture. By Fritjof Capra. Pp.464. US ISBN 0-671-24423-X; UK ISBN 0-7045-3054-6. (Simon & Schuster/Wildwood House:1982.) \$17.50, £9.50.

The revolution in the basic assumptions of physics brought about by Einstein and by the development of quantum theory has repercussions far beyond that discipline. One of the consequences of the new physics—that the mind of the observer is a necessary ingredient of the structure of theory—has not only returned terms like "mind" or "consciousness" to respectable scientific status; more far reaching in the views of a growing number of scientists, philosophers and historians of science it also undermines the mechanistic world view that originated with Bacon, Descartes and Newton.

While physicists gradually absorbed the new ideas in their own field, others spelled out the wider implications of these revolutionary changes for the entire scientific enterprise. Michael Polanyi, for example, argued for the subjective character of all knowledge (Personal Knowledge; Routledge, 1958); Michel Foucault in the Order of Things (Tavistock, 1966) demonstrated the dominance of epistemes, the communality of tagit basic assumptions in the most diverse sciences at given historical periods - in other words, the domination of world-views in science and their periodic changes; Thomas Kuhn, in The Structure of Scientific Revolutions (University of Chicago Press, 1962 and 1977), has made the notion of shifting paradigms fashionable.

To the extent that there is agreement among these authors and with others who write in the same key, all of this amounts to a clarification, perhaps a redefinition, of the aims of science: no longer is science conceived as cumulatively proceeding to the establishment of ultimate truth about a finite number of laws that govern a finite universe; no longer can science make apodictic statements about ontological essence since the laws of nature are not simply out there but anchored in the minds of men. Rather it is a conceptual enterprise resting on unproveable basic assumptions that have changed in the past and presumably can change again.

Physicists who have made their thoughts about the philosophical implications of quantum theory explicit seem to agree with this conception of science. But the mechanistic view still dominates and the search for discovering the real essence of the world continues. Paradoxically, perhaps, the "hard" sciences are now more open to admitting subjective meaning into their theories, while the "softer" life sciences, particularly aspects of biology, progress on the mechanistic road.

The two books here under review continue the debate with several new twists. Their virtually simultaneous publication, though obviously written independently from each other, must be understood as indicating that the lines of thoughts and arguments they present are gaining ground. Otherwise their truly amazing similarity would be hard to explain. Both have the same scope, beginning with the scientific revolution, proceeding to a careful analysis of the thoughts and contributions of Descartes and Newton, and ending with a sketch of

quite similar new and gentler world-views, that do away with the body-mind dichotomy and the idea of the control of nature in favour of an ecological perspective in which the distinctions between subject and object, value and fact are deliberately blurred. Both authors are deeply impressed by what they see as a convergence with Eastern philosophy (Yin-Yang, Zen Buddhism) of the ideas in quantum physics; both condemn the excesses of reductionism in favour of holism; both write for the general reader (otherwise I would not dare to review them) but in a scholarly fashion, with detailed notes and references; both regard Gregory Bateson's Mind and Nature (Dutton, 1979) as the most seminal modern work; both have the same critically respectful attitude to Freud, while preferring the development of his ideas by Carl Jung, Wilhelm Reich, Ronald Laing and Herbert Marcuse. Finally, both are centrally concerned with describing the misery of the modern world with its violence; poverty, consumerism, alienation, inflation, drug dependence. lunatic arms race and ecological destructiveness. In this last respect they are, of course, not alone. In contrast to the Meadows' Limits of Growth (Universe Books, 1972), with which they have otherwise much in common, they are cautiously optimistic because they see the new scientific world-view already emerging in North American culture.

Differences between these two books are largely matters of emphasis. Capra, a physicist, is particularly good in describing the development of physics from Newton to the present and is careful to point out which of the modern theories he adheres to are still controversial, for example, Chew's S-matrix theory. While he represents it in a language understandable to the layman and admits that he singled it out because it supports his broader views, it remains impossible for the non-physicist to understand its significance. Berman, a historian of science, writes most interestingly about Newton's psychological conflicts and complexities, and goes altogether more into psychological and political matters. While he, as much as Capra, admires Bateson whose thoughts and sources he describes in greater detail with particular emphasis on the principle of the essential incompleteness of knowledge, he also recognizes that some of Bateson's concepts are double-edged and could be exploited for totalitarian and anti-intellectual purposes; of which he strongly disapproves.

Two major common themes in these books deserve, however, critical comment. The first is the postulated link between the current malaise in the industrialized world and the mechanistic assumptions of Newtonian science; the second and related theme is the reductionism—holism issue.

A yearning to make coherent sense out of one's experience of being alive is probably universal; in Capra and Berman, both equally alive to the crisis-ridden state of the modern world and the state of modern science, their search for coherent meaning leads them to fuse these two realms into a world-view in which they are inextricably interdependent. Theirs is one interpretation of the past course of history and its possible future trend, but in the light of their own words they are honour-bound to admit that it is a subjective interpretation and that others are possible. One cannot take exception to their yearning for a better world nor to their description of the development of science. But there are good reasons to regard the link between them as not established. Human ingenuity in creating untold misery did not wait for the development of a mechanistic world-view. When Rome burned while Nero fiddled, when underoccupied soldiers in the fourteenth century turned brigands and plundered, raped and burned the French peasantry, devastating its agriculture, very different world-views prevailed. The holistic world-views that have for thousands of years dominated thought in the Far East have not avoided hunger, violence and overpopulation, nor the Cultural Revolution. Furthermore, the assumption that for the period with which these books deal one world-view permeated societies is questionable. People did not and do not regard themselves as machines, notwithstanding the existence of robots. In their search for meaning they have turned neither to Newton nor Niels Bohr, but increasingly in the United States to curious sects, pseudoreligions, creationism, blind imitation of Eastern practices, extrasensory perception, spiritual media and astrology all of them dangerous in their thoughtlessness as Berman explicitly recognizes, but all of them nearer to the advocated world-view than to mechanistic conceptions.

If the argument is, however, that it is not the general population but those who hold political and economic power who exemplify the Newtonian system and thereby run us into destruction, one could equally well argue that their many misdeeds are the result of sharing essential ingredients of the new view: all too many political and economic pronouncements blur fact and value, object and subject, just

- Paul and Anne Ehrlich's Extinction: The Causes and Consequences of the Disappearance of Species, reviewed in last year's Autumn Books Supplement by Kenneth Mellanby (Nature 294, 41; 1981), has just been published by the UK by Gollancz. The book was originally published by Random House in the United States. Price is £9.95, \$15.95.
- A paperback edition of Biological Energy Resources, by M. Slessor and C. Lewis (reviewed in Nature 283, 316; 1980), has been published by E. & F.N. Spon, price £6.95.

as Capra and Berman advocate.

Thus one of the principal themes in these two books, the link between mechanistic science and the state of the world, stands on shaky grounds.

The other — holism versus reductionism — is treated by both authors somewhat more gingerly; both are too sophisticated to deny the enormous successes of the reductionist approach in science, but both argue that in science and the world at large holism should now replace it.

To the question of reductionism versus holism Douglas Hofstadter has surely provided the final answer in his marvellous Ant Fugue (Gödel, Escher, Bach; Harvester, 1979) with the Zen word "mu" which means: unask that question. It all depends on what one wants to know. If one is after the chemical composition of cells, holism won't do: if one wants to know how people cope with the crises of life, no reduction to physiological brain-processes will provide an answer, even though brainprocesses are required in coping with life. The step from meaning to mechanism inevitably avoids the question to which an answer was originally sought. The meaningful whole is indeed different from its constituent parts and must be studied on its level, but unless the parts are properly functioning, wholes would collapse. So the parts, whether of human being, of ant colonies, of the environment or of the planetary system must be understood too.

It is true that many scientists still regard the reductionist approach as more "scientific", whatever that may mean. But the development of systems theories is already a powerful antidote to such narrow scientism; Capra and Berman furthermore quote many good minds (and some not so good) who are already grappling from a system's point of view with the central task of all scientific enterprises: to formulate significant questions and design ways to explore their implications. Whether such questions can be raised and tackled by relying on the poetic and mystical worldview of the unity of nature where everything is interdependent with everything else seems to me doubtful. The oceanic feeling of being at one with the Universe is a wonderful experience in moments of ecstasy. For the more pedestrian rational enterprise of science it must be replaced by asking questions of partial systems, small enough not to transcend the powers of the human mind.

Capra in his preface admits that with so large a scope he may have been superficial and simplistic when discussing many diverse fields of study, but he trusts that the whole of the book will be more than the sum of its parts. In contrast, this reader found many of the parts in both books informative and interesting, but the whole a bit muddled.

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Genesis of belief

John Maddox

Fundamentalism and American Culture: The Shaping of Twentieth Century Evangelicalism 1870–1925. By George M. Marsden. Pp. 306. ISBN 0-19-502758-2. (Oxford University Press: 1981.) £11.50, \$19.95.

LAST year's trial in Arkansas about equal time for creationism and evolution may have ended satisfactorily, but why was there in the first place a law for Judge Overton to declare unconstitutional? The wish to find out is a good reason for tackling this intricate but elegant piece of scholarship. Disappointment that there is no simple answer is overwhelmed by the richness of Dr Marsden's tale.

The book is an ecclesiastical history of the multicoloured and often warring denominations of American Protestantism in the half-century that ended in 1925 with the Scopes trial in Tennessee, Full-blown Fundamentalism was then only five years old and, Marsden says, never fully recovered from that defeat. Its end is symbolized by the death, on the Sunday after the trial ended, of William Jennings Bryan (counsel for the prosecution), once nearly President of the United States and afterwards, for a time, Woodrow Wilson's Secretary of State. But H. L. Mencken's savage obituary essay could not also serve as an epitaph for grass-roots Fundamentalism — there was too much of it. "Heave an egg out of a Pullman window and you will hit a Fundamentalist almost anywhere in the United States today."

By Marsden's account, Fundamentalism emerged from the reaction by Baptists and Presbyterians against the liberal theology taking hold in their denominations. But its origins lie in the "dispensational premillennialism" of the nineteenth century - the belief that the preordained history of the world specifies a final period (dispensation) of grace preceded by a second coming, itself the end-point of a period of social and moral degradation arranged by the Prince of Darkness. So the literal truth of the Bible, in particular the Revelation of St John the Divine, is the cornerstone of the faith, while saving souls against the second coming (Moody and Sankey in the United States, Spurgeon in Britain) is more important than social reform which might blunt the prophecy.

I had not known that the Fundamentalists own their name to the twelve-volume encyclopaedia of evangelical thought called *The Fundamentals* that was financed by the Californian oil-baron Lyman Stewart and was distributed free to more than 100,000 religious opinion-formers throughout the English-speaking world between 1910 and 1915. By then, the war between the premillennialists and the liberal theologians was well under way. There had been ructions among the Pres-

byterians about a proposed liberal revision of the "Confessions of Faith". Bible schools had sprung up, as had evangelists such as Billy Sunday — "I don't know any more about theology than a jack-rabbit knows about ping-pong, but I'm on my way to glory".

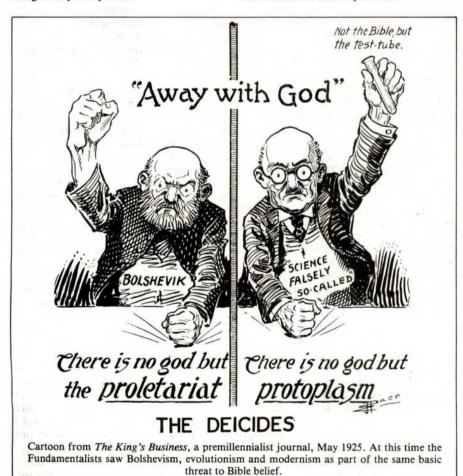
Marsden says that the Great War sharpened this conflict. Some premillennialists knew it to be the prelude to the Armageddon that had been prophesied. Bolshevism (1917) was another sign that the Prince of Darkness was at hand. The liberals, on the other hand, feared that such detachment would undermine the war effort. The social upheaval of demobilization, the financial crisis of 1919, the rise of gangsters and even the spread of cigarette smoking confirmed the premillennialists in their gloomy diagnosis. By 1920, both the Baptists and the Presbyterians were at loggerheads among themselves. The Fundamentalist crusade to purge the denominations of soft theology made the Scopes trial inevitable.

What explains the intensity of this dramatic conflict, centred, to begin with, in the north and not the south? Partly it was the nostalgic response to the final passing of the world order, signalled by the War. But Marsden persuasively explains how the tightly-knit immigrant communities of the early decades could have sensed each unfamiliar idea or unexpected event as a threat to the Protestant society they thought they had joined.

Politically, Fundamentalism was conservative (which is not the same as Republican) and fearful of an outside world seemingly rife with Bolshevism and Socialism. On balance, Marsden argues, Fundamentalism was not anti-science as such: indeed, it claimed to be the only scientific interpretation of the facts (the Bible). But evolution, "A string of guesses strung together", was a different kettle of fish. Marsden agrees that in the 1920s Fundamentalism was, for several interesting reasons, largely an American phenomenon. Perceptively, he asks what is to be made of the Fundamentalism now emerging in Islam and (in a footnote) in Ulster.

Marsden's history throws some light on those recent events in Arkansas even though 1981 is well outside his period. It explains why evolution is a unique challenge to Fundamentalism, and why Fundamentalism persists. For you cannot expect such a strong and recent tradition simply to melt away after a few set-backs in the courts. What Marsden does not for me sufficiently explain is why, in a republic in which church and state are constitutionally so separate, so many people have for so long invested so much energy and passion in such fierce and pointless arguments. "The God of your choice", it seems, demands a hard sell.

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Expressing science

Maeve O'Connor

Writing a Scientific Paper and Speaking at Scientific Meetings, 5th Edn. By Vernon Booth. Pp.48. (The Biochemical Society: 1981.) £2.50, \$6.

WRITING [maketh] an exact man, wrote Francis Bacon. How exact does it make scientists, already expected to be the most exact of men and women? And do they learn through writing to become not only more exact thinkers but, in turn, better writers?

Most editors would agree that although some scientists are superb writers and many are adequate, many more are imprecise, obscure, wordy or just plain careless. This is why journals tend to call for accuracy, simplicity and conciseness in their instructions to authors and why some editors devote hours to showing authors how to reach these goals. Editors and their requests for brevity are nevertheless often blamed for squeezing the life out of the socalled "literature". If blame must be assigned, however, we should direct it instead at educational systems that seem hard-pushed to produce literate graduates, never mind literate school-leavers. Yet scientists in particular, who must communicate their thinking successfully to other people if they are to help either society or their own careers, need some training in the art of presentation. Even in this age of cash crises, universities therefore ought to arrange appropriate courses. At present these are rare, but the return on the small investment needed could be enormous.

Until more courses become available, individuals could help themselves by studying one or two books on writing. Among these, Vernon Booth's booklet probably packs in the most good advice per square centimetre. This began life in 1971 as the Koch-Light Laboratories prizewinning essay. Dr Booth has revised and amplified the fourth edition (1977) and added eight pages on speaking at meetings. He does not claim to provide a complete text on how to write but simply to show readers how to avoid the mistakes he has met in scientific papers. He tells us what to do before writing, when to begin ("early") and how to get going ("Begin with the easiest section"). He gives useful hints on handling the various parts of a paper, and he covers all the commonest problems of literary style and punctuation in 12 succinct pages. In the section on speaking at scientific meetings he continues to administer sound advice, though little of this is particularly new. Like the rest, however, this section is pithily put and should be invaluable to busy scientists.

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Science in society, society in science

C.A. Russell

Between Science and Values. By Loren R. Graham. Pp.449. ISBN 0-231-05192-1. (Columbia University Press: 1981.) \$25.90, £14.40.

SCIENCE is about what is — what exists "out there" in the given world of nature. Values, however, are about what ought to be. They derive, not from ethically neutral scientific data but from the hunches of a Marx, the ambition of a Hitler, the Ten Commandments or even the Boys' Own Paper. At least, that is how the distinction is often made. On that view it is not surprising that the world between science and values has been frequently thought not to exist. Many people still believe this today, though it is a curious fact that they may arrive at such a conclusion from quite opposite directions. One group would deny the concept any validity because science and values are seen as overlapping aspects of one unified whole, each of which can expand to comprehend the other. There is no more meaningful an area "between" them than there is between the North Sea and the English Channel. Another group, however, would conceive of science and values as such different entities that they must be kept in watertight compartments and, since they must not be mixed, nothing could exist that had a little of each.

In this book such groups are labelled respectively "expansionists" and

The Strain and the Bond

She was a melancoli clone,
A prey to moods and shyness,
Who stayed at home, and grew alone —
A virtuous f-minus.

She loved another from afar: She idolized his gender. She little knew of H-fr (And less of Waring blender).

Responding to her coliform, And tempted by her colihood, The H-fr took her by storm (As willful f-rs would).

Beguiled by airy persiflage (A noxious kind of coltergeist), She caught a bout of coliphage; Succumbed to it; and lysed.

So, maidens, stay alone — and well — Remaining blithe and bonny, While other cultures go to Hell In coli matrimony.

This poem is taken from Ralph Lewin's *The Biology of Algae, And Other Verses,* recently reissued by University Press of America at \$6.75. Another taste of Professor Lewin's verse appears on p.506.

"restrictionists". The former hold that science and values did, and do, have much in common and, while in one sense there was no separate world "between" them, they were capable of the most potent interactions that could have consequences for human history of the profoundest importance. That view, rather than the opposite restrictionist one, informs this wide-ranging analysis of scientific controversy in the twentieth century. Graham begins by an examination of relativity and quantum theory, contrasting the metaphysical attitudes of Einstein and Bohr, casting a quizzical eye at the popularizing work of Eddington, and relating to their own cultures the scientific philosophies of the Soviet physicist Fock and the German Heisenberg. In the life sciences, the speculations of Bergson and Monod are followed by the rise of behavioural psychology and ethology. The author has some extremely important things to say about the cases of sociobiology and eugenics and the problems presented in biomedical ethics.

Historians of science will find much to admire in this book, not least its careful documentation, extensive bibliography and authoritative insights into problems that are rather inaccessible (as the history of Russian physics) or else are very recent. But the issues raised are not merely of academic interest; many of them are of urgent practical importance. While the author has no panacea to offer to those facing the dilemmas posed by genetic engineering, biomedical ethics and the claims of sociobiology, he suggests many useful pointers. And one clear conclusion emerges again and again: the inadequacy of restrictionism. Historical analysis repeatedly denies us the luxury of even contemplating the emergence of either science or values without the other. Expansionism is more conformable to historical reality, though prone to abuse and perversion; one need only cite the biologies of Nazi Germany and Stalinist "fraudulent Russia which were interpretations and misuses of science that could have arisen only in their particular political and social milieux" (p. 255).

By concentrating on recent science, and especially those branches concerned with man, Graham has established his case decisively. From now on behavioural psychology, genetics and other related areas of scientific enquiry will be seen by historians as inseparably involved in value-judgements almost from their conception onwards. This is a useful achievement, though not unexpected. It will be good news to those who over the past few years have assiduously sought to debunk "the myth of value-free science". They may, however, find some of Graham's other conclusions rather less welcome. Following

Toulmin and others he views science as a spectrum with the value-laden social and human sciences at one end, but with the largely value-free physical sciences at the other. His carefully documented casestudies offer some support for the view recently expressed (by D. M. MacKay) that the notion of universally value-laden science is an illogical extrapolation from the social sciences (where it is undoubtedly valid). Although his main purpose is to discover and demonstrate how science has affected values, the reverse process is considerably illumined by the author's narrative, and indeed the whole concept of value-laden science is complex enough to justify some analysis. It can mean at least five things.

First, it refers to values involved in choices made by individuals as to research career, subjects and modes of research, publication and so on, and by institutions as to whether to fund research and, if so, in what ways, and whether to exercise any control over it. In the last connection Graham concludes that a core-area of science should be exempt from such control and elsewhere much hard thinking needs to be done before exercising the control deemed necessary. No one would surely doubt that in this almost trivial sense science is always laden with values of individuals and society.

Second, science may be value-laden in terms of its theories. The author lists cases where the cognitive structure of science has been affected by systems as diverse as Puritanism, Naturphilosophie, monism, racism and Marxism, their distinctive values affecting the fabric of scientific thought. The different ways in which genetics developed in Germany and the Soviet Union during the 1930s illustrate particularly well the uptake of cultural values in the development of natural knowledge. His example of Copernicanism is perhaps less well-chosen since it may be doubted whether the decentralization of man was seen clearly by Copernicus as a demotion, whether Calvin ever did oppose the new system (p. 303), or even whether the "mountain of evidence" produced by Galileo was so regarded at the time (p. 362). But the essential point remains that Copernicus made a choice rather than a discovery, and that therefore values must have been present at the beginning. It is not going too far to suggest that most scientific theories are at least partly constrained by social values. That, however, is not to say that there is nothing to differentiate them in kind from (say) theories of politics or literature. The difference lies in the appeal to empirical data, and it is in this third area that the assaults on the concept of valuefree science are least successful. Given that "a real physical and biological world exists" (p. 25), empirical data and regularities will also exist independently of the ideology of the observer. Nowhere is this more true than in the physical sciences. Perhaps that is why Eddington could be so

sure of his restrictionist position, with his limitation of science to just "pointer readings".

The application of science to technology marks a fourth opportunity to seek for values, and they are not hard to find. Decisions as to whether and how to apply science inevitably involve valuejudgements, and few would quarrel with that assertion. A point of interest that does emerge from Graham's discussion of medical ethics is that strictly scientific knowledge plays a relatively small part in creating and solving the various dilemmas. The case of amniocentesis is instructive. Medical technology, much more than scientific research, has determined the dimensions of the problem, and the social climate is even more important.

The last kind of way in which science could be said to be value-laden is in its attempted extension and application to social problems as such. In this case it is much more true that science influences values than that it reflects them in its structure and practice. And here, in example after example, Graham demonstrates how readily this happens. He traces the story from Einstein (who, though a prolific writer on social matters, denied that science was directly related to values) to the sociobiologists' conclusion that all values are under genetic control - "the ultimate expansionism". En route he has much to tell of the ways in which science was used to justify ethical norms (as with Social Darwinism and capitalism) or even to explain them. He reminds us that several key figures, as Bohr, changed their views with time; he observes that early eugenics, far from being a tool of the Right was regarded in Weimar Germany as a Leftist deviation; and he cautions us against always supposing that "science is forcing changes in our values" (p. 267).

The author makes no attempt to conceal his own values. He emerges as a liberal and humane person, determined to see the best in ideologies with which he disagrees. Naturally some of his conclusions are highly contentious. Does it really follow, for instance, that dialectical materialism, whatever its merits, "has been damaged --probably beyond the point of salvage - by the fact that it is the political doctrine of an oppressive, nondemocratic state" (p.349)? And is restrictionism always antiscientific? What about Bacon and his two books of Nature and Scripture? And who is he that shall decide the issues of human genetics "without a priori commitments" (p. 255)? Nevertheless we can be grateful for an original, provocative and at times brilliant demonstration of the relevance of historical enquiry to critical issues of our day.

The second use of nuclear weapons

Laurence Martin

Nuclear Illusion and Reality. By Solly Zuckerman. Pp.154. UK hbk ISBN 0-00-216555-4; US 0-670-51822-0; pbk ISBN 0-00-216554-6. (Collins/Viking: 1982.) Hbk £7.50, \$10.95; pbk £4.95.

THE illusion which Lord Zuckerman mentions in his title, and which his book is chiefly devoted to exposing, is the idea that nuclear weapons could ever be used as a rational means of defence. A nuclear war would, he asserts, almost certainly become total, mutually annihilating, even if it began in a small way, which is itself improbable:

nuclear weapons may well be classified as strategic, theatre, and tactical, but these terms are meaningless if the use of one of them may mean the use of any.

In effect, then, nuclear weapons only serve to deter the use of nuclear weapons and Lord Zuckerman, while believing in the necessity for such deterrence, advocates what is usually called a "no first use" policy. Unilateral nuclear disarmament would expose the West to nuclear blackmail but, for all practical purposes, defence against aggression short of nuclear attacks must be provided by conventional forces. It is to those, argues Lord Zuckerman, in common with many voices now raised in the Trident debate, that our military energies should be vigorously directed. On the British nuclear force, he is a little ambivalent. He scoffs at the idea that British disarmament would serve as a useful example to others and, while regretting the resources diverted from conventional forces, he concedes the British force is an adequate deterrent to nuclear attacks on the United Kingdom. His most original reason for favouring the maintenance of the British and French forces is, however, that as small but effective deterrents they serve to demonstrate the superfluity of the huge forces maintained by the United States and Soviet Union.

There is clearly a good deal of plausibility in Lord Zuckerman's arguments and they are advanced with admirable lucidity. He pokes some shrewd holes in much that passes for strategic wisdom: pointing out for instance that those who cite the small CEP (Circular Error Probable) of missiles usually fail to consider the 50 per cent of warheads that will fall elsewhere, some with really gross errors; that tactical nuclear weapons have been widely distributed without any convincing doctrine for their use having been concocted even to the present day; that small nuclear warheads are not even very effective for many military purposes and that efforts to use them may consequently entail using large and devastating numbers. Nor can one fault his

lengthy explanation of what the effects of large scale nuclear war would be, though we are perhaps not lacking in earlier expositions of this theme.

For those already immersed to some degree in the nuclear debate, however. there are two threads in Lord Zuckerman's book that may be received rather cautiously. One of particular interest to readers of this journal is Lord Zuckerman's repeated attacks on the role the primary role it often appears - of science and scientists in promoting the "arms race". There is, of course, room for debate as to whether the international military competition that undeniably exists is so unlimited, and so useless, as the metaphor of race suggests. This debate is important, for the answer should influence our readiness to seize on such remedies as are proposed.

So far as the specific role of science is concerned in stimulating the competition, whatever its intensity, there are actually two kinds of scientists in Lord Zuckerman's world. The majority appear to be rather lower grade people, usually called "technicians", who work in military research establishments and foist innovations on the military and the politicians. There is also, however, a small elite of scientific advisers - in Britain including Lord Zuckerman himself, in the United States his friends Jerome Wiesner, Herbert York, Franklin Long and so on who see the uselessness of it all but whose advice never seems to have been taken when anything wrong was done. One can only admire the resolution with which they nevertheless clung on to the job.

The lesser kind of scientist is apparently the fundamental cause of our military predicament: "At base, the momentum of the arms race is undoubtedly fuelled by the technicians in governmental laboratories and in the industries which produce the armaments". There have been several recent studies of this problem, chiefly in the United States, and it undoubtedly deserves even more thought. Neither militarist nor keen disarmer should tolerate military developments that serve no useful strategic purpose. Doubtless some weapons do get made because they are "there" and sometimes, perhaps, a weapon emerges not as the result of a military specification but because a diversified technological process throws up the necessary components: possibly the cruise missile is such a case, coalescing from disparate developments in guidance, propulsion, image suppression and so on.

But this does not mean that the cruise missile is a useless or necessarily deplorable weapon or that its production is the result of spontaneous generation rather than rational strategic decision. Lord Zuckerman seems to regard the MIRV as such a

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technology- rather than strategy-led growth. Yet we know that the production of MIRVs was the subject of prolonged, agonized and explicit debate. It is surely going too far to absolve the political leadership of responsibility and assign it all back to the "technicians". It is interesting to note that in its latest Yearbook, the Stockholm International Peace Research Institute flatly repudiates the "driven by technology" thesis and affirms that the political system gets the weapons for which it calls.

Lord Zuckerman does not make very clear what he believes the main danger of the technology-driven arms race to be. Does sheer quantity of weapons make war more likely? There is no definite statement of this. It is not, apparently, that lesser quantities would do much to limit damage, for Lord Zuckerman is adamant that much smaller nuclear forces than the present ones would serve to wreak disaster. But dangerous Lord Zuckerman certainly believes the present military confrontation to be, and he despairs of survival if the East-West competition is not moderated by arms control before the end of the century. Obviously weapons secured for no logical strategic reason would be a waste of money. Reading more between than on the lines, however, it would appear that perhaps the main danger Lord Zuckerman perceives is that an unjustified reliance on nuclear elements in NATO strategy might lead to the use of nuclear weapons despite the fact that it would be irrational to do so.

Not since the first contradictions of the idea of "massive retaliation" has it been possible to deny the reality of this danger, paralleled as it is, in Kennedy's famous phrase "holocaust or humiliation", by the danger of political defeat if the threat to use nuclear weapons proves hollow. Lord Zuckerman's cry for us to look to our conventional forces, and to recognize that nuclear deterrence requires an adequate underpinning of lesser forces, is amply justified.

It is here, however, that an all-too-brief reference to a second debatable theme in the book should be made. Lord Zuckerman is an advocate of "minimal nuclear deterrence" combined with conventional defence, and almost totally rejects any idea that nuclear weapons might be used in limited ways for "warfighting" or to introduce an "intra-war" level of deterrence by nuclear means. His one concession is to admit that

it is just possible that one side might not reply in kind, say to a nuclear 'shot across the bows' or to the attempted 'discriminate' use of nuclear weapons, or even to a first strategic strike.

But he goes on to say that in practice retaliation would be unrestrained for psychological reasons and for lack of technically adequate command and control. When citing with disapproval Dr Harold Brown's statement as Secretary of Defense — as an earlier "scientific adviser" Brown gets good marks — that a

flexible strategy should "leave open the possibility of ending an exchange before the worst escalation and damage had occurred, even if avoiding escalation to mutual destruction is not likely", Lord Zuckerman says only "the final ten words of this statement are those that matter".

Whether even "not likely", if accurate - and certainly it is hard to be optimistic about such a juncture - justifies making no provision at all for "controlled" nuclear action in such a contingency is, and is likely to remain, a vexed and probably the most important strategic question of our advancing nuclear age. It is good to have Lord Zuckerman's firm view to ponder. The logic of his position leads to his "no first use" policy. But we should not accept this without first dwelling on two or three difficult questions. What is the effect of a "no first use" policy in a coalition where the main and, if many had their way, the only nuclear forces belong to a distant



Lord Zuckerman - a view to ponder

ally? If nuclear attack on Western Europe may not be regarded as a "first use" on the United States, should the United States have an identifiable separate level of "retaliation" to deter in such cases? And if a future war in Europe is given a virtually assured "conventional" character, by a "no first use" policy, would the calculation facing a would-be Soviet aggressor—"Can I win a conventional war in Europe"— be dangerously different from the one facing him today—"Can I afford to start a war in which nuclear weapons will probably be used"?

In practice, the balance of deterrence in Europe is probably stable over a wide range of strategic postures, because the stakes are so high and the all-pervasive aura of nuclear fear is so daunting, whatever the explicit strategic doctrines. But in the long run, underlying assumptions about how nuclear deterrence is related to overall deterrence must have profound effects on how states prepare in peacetime and might behave in war. For those who think such questions important, Lord Zuckerman's book offers a provocative and forthright essay well worth the reading.

Laurence Martin, Vice-Chancellor of the University of Newcastle upon Tyne, was Professor of War Studies at the University of London from 1968 to 1977. He delivered the 1981 BBC Reith Lecture, "Armed Force in the Modern World".

Quantum theory: certainty in uncertainty

P. W. Atkins

The Cosmic Code: Quantum Physics as the Language of Nature. By Heinz R. Pagels. Pp.370. ISBN 0-671-24802-2. (Simon & Schuster: 1982.) \$17.50.

THIS is a book aimed squarely at the general reader with the intention of conveying not only the excitement of modern science but also the revolution in our comprehension of the world that accompanied the introduction of quantum theory. There are three parts: an account of quantum theory; a description of modern particle physics; and a brief personal reflection on the nature of physical laws. The material is presented nonmathematically and will be accessible to anyone who is interested in what the author rightly regards as the nonpareil of this century's social and cultural events. The text itself is characterized by the author's almost boyish eagerness to communicate his enthusiasm, and is sometimes so anxious to please that it trips over its own naivety ("Most physicists enjoy the outdoors") to the point, in one place, of ascribing the well-known allegory of Paley's watch to a speaker at a recent conference.

The most important section is the first, which occupies about half the book. It is woven around the biography of Einstein and his transition from being a revolutionary innovator to one who lost "his hot-line to the Old One" and refused to accept that determinism was dead. The intention of the section, apart from emphasizing a moral, is to present an exposition of quantum weirdness; the loss of objectivity, its replacement by an observer-created reality and the rejection of determinism - "the quantum theory is a theory of an instrumentally detected material reality" (p.99). Underlying (if not stemming from) this intention is an opposition to material reductionism (a programme that "cannot be carried out", p.135). The basis of this view is an acceptance of the Copenhagen interpretation of quantum mechanics with its emphasis on the meaninglessness of a concept until its mode of measurement has been defined. Pagels adopts the party line on this issue, and as such does a very good job. His argument leads, convincingly, to the ultimate quantum weirdness that "human intention influences the structure of the world" (p. 95), and the view that "quantum reality" is statistical.

Convincingly, that is, until one stops to think. In my view, for what it is worth, determinism entered physics with the uncertainty principle, and the principal confusion pervading so much interpretative commentary on quantum mechanics (and finding such lucid expression in this book) is the failure to distinguish between measurement and

specification. I should explain what I mean. With quantum theory we first encountered a constraint on our description of the world. Classical mechanics attempted unknowingly to be overcomplete in its specification; quantum mechanics is the first theory we have (and possibly the last we shall need) that respects nature by refraining from imposing a description too strong for it to support. Quantum mechanics is fully deterministic in the sense that the evolution of a state under the influence of a hamiltonian is perfectly well-defined; it ceases being deterministic only when we insist upon reverting to an overcomplete discussion by asking questions that classical mechanics has conditioned us to believe are answerable or when, after we have prepared a system in an eigenstate of one observable, we seek to predict the outcome of another observation that classical mechanics has conditioned us to expect but which quantum mechanics proscribes. No wonder our predictions then squirt out in all directions! Quantum "randomness" is nothing more than this reaction to false expectation. Likewise, the strict Copenhagen requirement of "meaningless until measured" is a confusion between a mode of interpretation and a manner of exhibiting self-consistency (or at least of constructing thought experiments to show that there is no inconsistency). This viewpoint is consistent with Einstein's whose views, I suspect, have been overborne by the appeal of an interpretation that hangs on to classical modes of thought. This emphasizes yet again the delicacy of the balance in the dictum (p.67) that "physicists are conservative revolutionaries pseudoscientists lack that commitment to existing principles".

My position then is strict determinism (or better, neodeterminism, because it must not be confused with classical determinism) showing itself as indeterminism only when false, culturally conditioned, classical questions are asked. Hence, in my view, Pagels has focused on a wholly misleading and fallacious interpretation which cannot fail to imbue his readers with a false impression of the nature of "quantum reality". I have to admit, though, that he has the forces of neoconvention ranged on his side (which does not mean he is right), and readers of this engaging book will leave it with a clear grasp of a conventional and widely accepted interpretation.

I have concentrated on a single aspect of the book, but one that lies at its core. There are many other remarks with which the careful reader will probably disagree. For instance, I could criticize at equal length the remarks to the effect that differences between macroscopic and microscopic phenomena are qualitative and not merely quantitative (p.128), that historical events are not reducible to individual acts on the part of human beings (p.131), and that quantum reality is statistical and therefore outside mathematics (p.337). There is also a peculiar blindness to the technical meaning of a "selfish" gene but not to its analogue "charm". There is also much to admire, especially the author's manifest deep concern to communicate.

P. W. Atkins is a Fellow of Lincoln College, Oxford, and author of The Creation (W. H. Freeman, 1981).

Getting JET off the ground

John B. Adams

A European Experiment: The Launching of the JET Project. By Denis Willson. Pp.181. Hbk ISBN 0-85274-543-5; pbk ISBN 0-85274-549-4. (Adam Hilger/Heyden: 1981.) Hbk £10.50, \$23; pbk £6.95, \$15.50.

EVERYBODY who is concerned in Western European affairs should be interested in this account of "a European experiment". Opponents of the European Community system will find in it ample confirmation of their criticisms; supporters will see in it a triumph of the European spirit over national self-interest. For those who are convinced that international action is now the only way of carrying out major scientific enterprises in Europe, it will come as a sobering reminder of how very difficult it is to reach agreement at the European level.

The subject of the book is the launching of the Joint European Torus (JET) project, the latest and largest experiment in the world-wide effort which started back in the 1950s to exploit nuclear fusion reactions as a useful energy source. To make this dramatic story (or lamentable comedy as it was once described) intelligible to the layperson, the author has first to explain something about plasma physics, the general history of nuclear fusion research and the complexities of the European Community system, all of which he does with commendable clarity. Having provided the reader with the essential background information, he then describes the specific problems presented by JET.

The principal difficulty that arose in the launching process and the reason for the two-year delay in the approval of the project was the problem of where to locate it. After the failure of the Community to agree to a common nuclear fission reactor programme and the consequent disarray this caused to the Euratom laboratory at ISPRA; which was set up to carry out that programme, the Commission devised a



JET takes form — the eight limbs are part of the transformer core of the machine, currently under construction in the Torus Hall at Culham.

different method for pursuing nuclear fusion research in Europe. This was to set up a coordinated research programme using the existing national fusion laboratories rather than to try to bring all the work together in a single European laboratory. This method proved acceptable to the national laboratories and to the Governments concerned, and it worked very well until the JET experiment came along. Because of its large size and the need to engage most of the national laboratories in its design and construction, it did not easily fit into the established pattern. A new system was adopted, called a Joint Undertaking, which made JET a common European enterprise. But there was no European fusion laboratory in which to build it.

The Commission first suggested that JET should be built at ISPRA where there were resources then under-utilized. This proposal was opposed by many of the fusion scientists who feared that the project would be seriously handicapped by the absence of fusion expertise at ISPRA and by its previous history. It was also opposed by several member states, who saw JET as a desirable acquisition and who proposed alternative sites for it on their own territories. The scene was thus set for a first-class battle for the JET site in which all of the many levels of the Community became involved, and it is this battle which forms the main drama of the book.

Denis Willson describes these events, blow by blow, with great objectivity and with refreshing frankness which makes his book a very valuable account of the trials and tribulations of those who have the need and the courage - to launch international projects in Europe. It may even be used to avoid similar problems arising again in the future but, as the author points out, the Governments in Europe still have no agreed way of resolving such problems and it is difficult to see how they will acquire one as long as they insist on unanimity (which, incidentally, they had finally to forego to reach a decision on JET). But, despite all the difficulties, JET was finally approved and is now under construction next to the British nuclear fusion laboratory at Culham where it appears to be going very well.

Similar dilemmas faced by CERN, the European Organization for Nuclear Research, are occasionally quoted for comparison but it is not mentioned that it took CERN much more than two years to reach a decision on where to build its SPS machine. This problem was finally resolved

when the scientists involved agreed to reshape that project and themselves proposed the only site where it could be built—something which the scientists in the national fusion laboratories were apparently unable to do for JET.

This book is a well-written and thoughtprovoking account of a European experiment in all senses by someone who played a key role in the launching of JET and who had access to all of the documentation. It can and should be read and digested by all serious-minded citizens of Europe — politicians, civil servants, scientists and laypersons — and it will surely be read and noted by similar people in other regions of the world.

Sir John Adams, currently at CERN, was Director of the Culham Laboratory from 1960 to 1967.

Space for science?

John Noble Wilford

Beyond the Atmosphere: Early Years of Space Science. By Homer E. Newell. Pp.497. ISBN 0-80-607146. (NASA Scientific and Technical Information Branch: 1981.) Hbk \$12.50; pbk \$9.50.

FROM the start, in 1958, the National Aeronautics and Space Administration and the scientific community have had an uneasy and ambivalent relationship. It was probably unavoidable. For all their shared interests in exploration, they approached the space age with different priorities and expectations. Scientists saw in NASA a new source of research funding and of opportunities to study Earth, the Solar System and the Universe. While NASA was not ungenerous in its early dealings with scientists, its administrators marched to a different drummer. Given their political mandate, which was to establish the United States as the pre-eminent space power, the NASA managers accorded highest priority to projects aimed at "catching up" with and eventually "beating" the Soviet Union. Scientists had no choice but to accept a secondary role in the Apollo Project, for example, and to live with a relatively small share of the space budgets, now as then. Tensions between them and NASA have been an inevitable consequence.

The potential for conflict has, if anything, risen since the Apollo days. Even at the height of the Apollo mobilization, not a year went by without the initiation of a new space-science project. A small share of ample budgets paid for a lot of science. Post-Apollo budget reductions, however, coupled with inflation and the expense of the space shuttle, have now squeezed space science to the point of near-paralysis. New projects are rare, and existing ones are forever being threatened with deferral or cancellation, a fate that recently befell the Venus Orbiting Imaging Radar Mission and the American half of the International Solar Polar Mission. Only desperate lobbying by scientists, led by Carl Sagan, saved the Galileo Project from extinction this year.

In this context of uncertainty verging on despair, scientists will read Homer E. Newell's Beyond the Atmosphere with a sense of nostalgia for the early days of space science. Dr Newell, who retired in 1973 as NASA's associate administrator, bore much of the responsibility for mediating NASA's relationship with scientists so that a vigorous space-science programme was possible. He is a scientist himself who brought to his job considerable experience in managing Government science undertakings. His book is another in a series of histories prepared under NASA auspices, which means that it is, in a sense, "official history", though somewhat more candid than most works of this

Much of the book reviews the origins of space science as a part of NASA's operations and the achievements of NASAfinanced research from 1958 to the mid-1970s. Space science, the way Dr Newell uses the term, is the multidisciplinary pursuit of a knowledge made possible or significantly aided by rockets and spacecraft. The breadth of the field as it evolved is impressive: geodesy, meteorology, atmospheric and ionospheric physics, magnetospheric research ("a genuine product of the space age"), lunar and planetary science, solar studies, galactic astronomy, relativity and cosmology as well as the life sciences and exo-

Dr Newell acknowledges that the expanding perspective afforded by spaceflight has yet to produce any scientific revolution. But he writes with understandable pride of the "continuing harvest" of knowledge from space science, notably in the earth and planetary fields:

No longer was the geophysicist confined to a study of only one body of the solar system. No longer was the study of the planets solely a venture of the astronomers. The dearth of new data that had led planetary studies into the doldrums and even disrepute... gave way to a sudden flood of new information that reawakened the astonomer's interest.

Other exciting developments included the effect on astronomy of space observations in the hitherto hidden wavelengths and the

discovery of Earth's magnetosphere, not suspected beforehand.

For those scientists who have worked in NASA projects, or expect to, Dr Newell's concluding chapters will be of greatest interest. They deal with "the real world of budgets and finances". They seem at times to be a plea for more understanding by scientists of how competitive are the claims for NASA's favour.

Dr Newell is perhaps still too much the diplomat to render a critical historical treatment of the struggle between scientists and engineers within NASA and between unmanned and manned programmes. Such struggles persist to this day. The author, taking note of bitter feelings among scientists towards the manned programme, does remind them that even if they had succeeded in reducing or cancelling a project such as Apollo, there was little likelihood that much, if any, of the money saved would have been diverted to them. To the contrary, he observes, science generally benefited from Apollo by riding on its coattails for such lunar science endeavours as Surveyor and Lunar Orbiter. Moreover, he contends that scientists should have been grateful that they had a civilian agency like NASA rather than having to depend on military suppport, as had been the case in the pre-Sputnik days.

As for the future, Dr Newell proffers cautious optimism about the place of space science in NASA's operations, or at least he puts the best light on post-Apollo developments. Out of the "searching scrutiny" in the early 1970s, he writes, "emerged an acceptance of a continuing role for the agency in which science, applications, and exploration would all play a part". Moreover, the author says, NASA's position became "intrinsically stronger" because it is now "freed at last from an uneasy dependence on a passing sense of urgency over the nation's technological strength relative that of the USSR".

A failure of the book is the absence of any critical evaluation of the structure for the support of space science as it has evolved. Would space science be served better if it could count on several major sources of funds, not just NASA?

Dr Newell does advise that scientists must scale down some of their expectations if they are to win continuing support from NASA. These words are only now being heeded. Resistance by recent Administrations to large, new projects — the expensive planetary missions in particular — has prompted scientists working with NASA to shift their thinking to more modest goals.

Accordingly, the Space Science Exploration Committee, formed by NASA last year and chaired by Noel Hinners, Director of the National Air and Space Museum, is devising a new strategy. Missions with limited, specialized scientific objectives, employing lower-cost, standardized spacecraft, would be emphasized. Major undertakings, on the scale of another Viking or Space Telescope, would have little or no

place in planning for the foreseeable future.

It remains to be seen how acceptable this modest strategy will be to NASA's managers or the Reagan Administration. A review of the American space programme, being prepared now by the White House Office of Science and Technology Policy, will presumably have more influence on the future course of NASA than any group of scientists. If the Administration's pronouncements are any indication, the review will deal almost exclusively with the space shuttle and policy regarding NASA's jurisdiction vis-à-vis the Department of Defense. Meanwhile, the engineers in NASA are drawing up plans for another big project, the manned space station, that would probably absorb any funds released as a result of the shuttle's completion.

This can only give scientists new reason to be uneasy. The entire American space programme is in a state of flux, with the ascendancy of the Pentagon in space affairs and the re-ordering of NASA's goals and responsibilities. It is in times like these that space scientists may find themselves wishing Homer Newell was still in a position to attend to their interests within the chambers of NASA.

John Noble Wilford is a science correspondent of The New York Times who covers NASA. His most recent book is The Mapmakers (Junction Books/Knopf, 1982).

Heroes of AI

Richard Gregory

Science Observed: Essays Out of My Mind. By Jeremy Bernstein. Pp.376. ISBN 0-465-07340-9. (Basic Books: 1982.) \$16.95.

This is a personal account, in 17 essays, of recent highlights of physics and of artificial intelligence. The pieces are written in lively style by Jeremy Bernstein, a professor of physics and author of several well-known books, including A Comprehensible World, (1961), The Analytical Engine (1964), Einstein (1973), Experiencing Science (1978) and Hans Bethe: Prophet of Energy (1980). He also writes on science for the New Yorker.

Like ancient sagas, these essays are based on heroes, on men of science confronting the unknown and sometimes each other. Chief among them are Marvin Minsky, Einstein, Robert Oppenheimer, Schrödinger and Harold Furth. Others lie in the shadows, seen with fleeting ambiguities: Ernst Mach — father and disowner of relativism. Charles Babbage—grandfather of artificial intelligence, who set out to weave algebraic patterns with his

analytical engine, just as the Jacquard loom weaves flowers and leaves. Babbage spoke most clearly through the voice of Ada Augusta, Countess of Lovelace, rejected daughter of the poet Byron. And Alan Turing — father of artificial intelligence, who dared to oppose the wit of men against machines, to see if under their disguises men and machine are the same. (Or is oil thicker than blood?)

The first principal hero is the guiding genius of artificial intelligence at MIT, Marvin Minsky. Indeed he is, as I know by acquaintance and not only description, all that Jeremy Bernstein claims. With his equally brilliant colleague John MacCarthy, now at Stanford, he started the AI enterprise in the United States in the late 1950s, and they invented its name. "Artificial intelligence" is philosophically better than the rival "Machine intelligence", for if brains are intelligent machines the colours may get confused.

Minsky is a compelling writer as well as a delightful companion and true inspirer of students. Disarmingly, he dislikes the activity of programming, preferring to think and discuss and write in words. Of intelligence, Minsky wrote in 1961 (quoted on p.54): "To me 'intelligence' seems to denote little more than the complex of performances which we happen to respect, but not understand". And, "Programmers, too, know that there is never any 'heart' in a program. There are high level routines in each program, but all they do is dictate that if such and such, then transfer to such and such a subroutine". And, "When we look at the low-level routines, which actually 'do the work', we find senseless loops and sequences of trivial operations, merely carrying out the dictates of their superiors".

There is much of interest here on the history of computing, with figures of the sizes and costs and weights and energy consumptions, as well as the speed and power of ancient and modern computers through their generations. It is indeed astonishing that ENIAC, which in the late 1940s was the most complicated electronic device ever built, was a hundred feet long, ten feet high and three deep. It consumed 140 kilowatts, to handle twenty ten-digit "words" in its memory. On this scale, one could not get a present-day home computer into the house, or even the street.

Jeremy Bernstein is a scientist writing for scientists. He assumes that one knows about Mach's principle and Newton's bucket; and he also comments economically and without gush on such matters as the idiocy of using troops to test the effects of atom bomb blast and how well they would stand up psychologically to nuclear war. These are extremely readable sagas of our age; and they are well worth reading.

Richard Gregory is Professor of Neuropsychology and Director of the Brain and Perception Laboratory at the University of Bristol.

What skulduggery?

Steve Blinkhorn

The Mismeasure of Man. By Stephen J. Gould. Pp.352. ISBN 0-393-01489-4. (W.W. Norton: 1981.) \$14.95, £9.95.

WITH a glittering prose style and as honestly held a set of prejudices as you could hope to meet in a day's crusading, S.J. Gould presents his attempt at identifying the fatal flaw in the theory and measurement of intelligence. Of course everyone knows there must be a fatal flaw, but so far reports of its discovery have been consistently premature.

The theme of this particular book is that since science is embedded in society, one must expect to find the prejudices of the age presented by scientists as fact. Most authors, given such a theme, would be content to document and catalogue instances in support of the proposition. Gould, however, goes one better by writing a book which exemplifies its own thesis.

It is a masterpiece of propaganda, researched in the service of a point of view rather than written from a fund of knowledge. For the best propaganda requires not the suppression or distortion of facts but their careful selection, emphasis and juxtaposition. So, in a work which declares its concern to be with the notion of intelligence as a single measurable "thing" in the head, we find that two-thirds of the argument is given over to a careful reworking of early attempts to establish craniometric and anthropometric criteria of intelligence, and an admirably disturbing account of the Gadarene rush to press IQ tests into the service of social engineering in the USA in the first half of this century. As Gould rightly emphasizes, many of the uses to which tests were put made mockery of their original purpose.

Ottery

A fitter fits;
A cutter cuts;
And an aircraft spotter spots;
A baby-sitter
Baby-sits —
But an otter never ots.

Though sinners sin
And thinners thin
And paper-blotters blot;
I've' never yet
Had letters let
Or seen an otter ot.

A batter bats (Or scatters scats); A potting shed's for potting: But no one's found

A bounder bound

Or caught an otter otting.

From The Biology of Algae by Ralph Lewin. See p.500 for details,

The final third of the book is the attempt proper to debunk the notion of general intelligence as arising specifically in the school of factor analysts starting with Spearman. But by this stage the reader has been presented with sufficient examples, sufficiently carefully examined, of racial and social prejudice in the work of scientists, of distorted data, fudged analysis and twisted interpretation as to the inexpert might establish a necessary connection. Add to that the soft target of Cyril Burt. some rather inaccurate observations on the role and effects of the 11+ examination system in Britain and a remarkably detailed account of antique methods of factor analysis, and you have all the makings of a lively, plausible, opinionated and zesty potboiler.

But verbal fluency is no substitute for good arguments in the long run. The substantive discussion of the theory of intelligence stops at the stage it was in more than a quarter of a century ago. Consequently there is no account of attempts to characterize the psychological nature of general intelligence, no indication that multivariate methods have progressed beyond Thurstonian techniques, no discussion of the effects of ageing, of brain damage, of compensatory programmes, no account of modern behavioural genetics, of heritability studies other than Burt's, no hint of the current interest in cybernetic models or recent attempts by experimental cognitive psychologists to account for psychometric findings.

Gould even gives a perfectly straightforward account of what heritability would and would not mean in terms of the modifiability of intelligence, but fails to point out that such arch-hereditarians as Eysenck and Jensen have published essentially identical accounts. One is, presumably, meant to conclude that adherence to the notion that there is a measurable single dimension of intelligence necessarily involves the kind of radical nativism which was prevalent in times when genetic theory and statistical methods were in their infancy.

But this is a book with a double punchline. Or to put it another way. Gould performs the remarkable trick of pulling the rug from under his own feet whilst appearing to stand stock still. For not only does he propose a totally unobjectionable definition of intelligence ("the ability to face problems in an unprogrammed . . . manner"), which, far from being novel, is a nice rewording of a definition proposed by Cattell in the context of a heavily factoranalytic theory and something of a commonplace amongst the intellectual heirs of Spearman, he even proposes essentially craniometric criteria of neoteny as a basis for the adaptability of Homo sapiens, and produces a photographic comparison of adult and infant chimpanzees with the remark that "if a picture's worth a thousand words . . . ".

The truth of the matter is that Gould has

nothing to say which is both accurate and at issue when it comes to substantive or methodological points. His "fatal flaw" (the purported dependence of the notion of general intelligence on details of factor analytic technique), the unsupported assertion that "disadvantaged" groups always perform worse on IO tests, his strictures on the application of the notion of intelligence across species boundaries, his attempt to link the use of IQ tests in Britain with a rigid class structure, all have the routine flavour of Radio Moscow news broadcasts when there really is no crisis to shout about. You have to admire the skill in presentation, but what a waste of talent.

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Beyond selfish genes

Sydney Brenner

The Extended Phenotype: The Gene as the Unit of Selection. By Richard Dawkins. Pp.307. ISBN 0-7167-1358-6. (W.H. Freeman: 1981.) £9.95, \$19.95.

This book, as Richard Dawkins states in his first chapter, "is a work of unabashed advocacy". It does not put forward new theories or new facts, but it advocates a special way of looking at living things and the worlds they inhabit.

This point of view, called the extended phenotype, is a development of the ideas discussed in his earlier book The Selfish Gene where, it may be remembered. Dawkins aimed to dispose of the organism as the unit of natural selection. He argues that this unit is the gene, or, rather, entities which are called germ line replicators, of which genes are the most important examples. Adaptations are for the benefit of these elements and not for anything else. Of course, it is admitted that these replicators are useless by themselves (you can buy them in bottles as chemicals called DNA) because selection judges them only by their effects in other entities, called vehicles, which the replicators inhabit. Biologists have given one such discrete vehicle, the organism, a special role as a unit of structure and function and it is this hold that Dawkins wants to break. He claims that replicators have extended phenotypic effects, reaching out beyond the boundaries of the organisms where they happen to be lodged into the world outside, even entering other organisms. He wants to dissolve organisms:

We see through them to the replicating fragments of DNA within, and we see the wider world as an arena in which these genetic fragments play out their tournaments of manipulative skill. Genes manipulate the world

and shape it to assist their replication.

Later he speaks of the

image of a turmoil of selfish replicators, battling for their own survival at the expense of their alleles, reaching unimpeded through individual body walls as though these walls were transparent, interacting with the world....

The extended phenotype is certainly a big idea and it is pressed hard in dramatic language. But does it help to view genes as active agents, manipulating, reaching, battling? I think it only obscures the essential feature of living organisms which distinguishes them from all other complex natural systems and which gives them the capacity for evolution. It is that they are propagated by passing on not themselves but an internal representation of themselves in their genes. Exactly how this correspondence is implemented is what most biologists study; knowing it must be important if only for understanding what genetic variation can generate. To a molecular biologist, all the stuff around the genes is anything but transparent; it is hard • to see the replicators through the fog of complicated machinery, nor do they reach out so easily as Dawkins wants them to.

Dawkins still has to come grips with this central issue. In a section on the poverty of preformationism he would like to make a distinction between the central dogma of molecular genetics and that of embryology. The former states that information in protein cannot be translated back to nucleic acids: the equivalent in development is that "bodily form and behaviour may not be translated back into proteins". I think we agree that the former is a fact, not a question of principle, but why does Dawkins want to make the latter a principle and not simply an equivalent fact? After all, in the end we shall need to explain the effects of genes on bodily form and behaviour in terms of proteins; that is the only way for us to reach into organisms and for genes to reach out into the world. The molecular, cellular and organismic grammar connecting genes and phenotypic effects is the heart of the matter and the only way to explain how random changes in DNA can produce all the sense we observe in the living world.

Organisms exist. Even Dawkins realizes that he needs to bring back individual organisms. His extreme position has forced him into calling the organism a "phenomenon that needs explanation", and he offers only a summary sketch on the last page. He says:

It has paid replicators to behave gregariously. The phenotypic power by which they ensure their survival is in principle extended and unbounded. In practice the organism has arisen as a bounded local concentration, a shared knot of replicator power.

Sounds terrific, but what does it mean?

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The whys (and wherefores) of behaviour

Aubrey Manning

Ethology: Its Nature and Relations with Other Sciences. By Robert A. Hinde. Pp.320. Hbk ISBN 0-19-520370-4; pbk ISBN 0-00-636237-0. (Oxford University Press/Fontana: 1982.) Hbk £9.20, \$15.95; pbk £2.95.

ETHOLOGISTS are no longer a distinctive breed; indeed the days when one could recognize them from their background and framework of theory were quite short. Large parts of the original theory built up from Lorenz's dichotomizing between the "innate" and the "acquired" in behaviour, and the energy model of drives, have been abandoned. What has endured is an approach to the animal in its natural environment which is highly productive and infectious. When Lorenz and Tinbergen built up their schools following the Second World War, ethology came as an invigorating breeze across the arid fields of so much of the white rat experimental psychology of the day. Its influence has persisted and spread widely into other areas of behavioural science.

Robert Hinde's book sets out to give an account of modern ethology and to examine this influence. He succeeds well and this is both a useful and a stimulating book. I particularly liked the brief historical introduction to each section. Since modern ethology only goes back about 30 years and Hinde himself has been a highly influential figure over most of this period, we get a direct picture of an emerging discipline as it interacts with others already established.

The plan of Hinde's opening section on "core ethology" derives from Tinbergen's classic paper of 1963, "On the Aims and Methods of Ethology". This was a landmark in the history of the subject and it represented a clear break of approach between Lorenz, who then had just published a reaffirmation of his own unchanging views on innate behaviour, and Tinbergen, who saw far more clearly that the old dichotomies (nature or nurture etc.) would no longer do. Tinbergen enunciated four types of question in relation to behaviour: those concerning its immediate causation, its development, its function (in the sense of survival value) and its evolution. Hinde has chapters covering each of these topics - he calls them ethology's four "whys". In fact they form a very good introduction which both covers some of the classic ethological studies and is also right up to date.

There is no introduction to this new Masterguides series itself and I am uncertain what audience they seek. Other titles include Religion, Law and Social Anthropology, so I imagine that "the educated layman" is once more in mind. Hinde is certainly not writing at an elementary level, nor can he bear to let

first-stage simplifications stand. Readers without some behavioural background will find parts rather heavy going and there are one or two over-complex diagrams. References to the literature abound on almost every page, and no doubt or inadequacy of the evidence is left unexposed. Just these latter qualities, of course, will commend the book to the serious student. Its main body is Hinde's assessment of the influence which ethology has had upon related fields in biology and the human social sciences. They cover a huge range, from ecology to psychiatry, and are consistently illuminating. Hinde's coverage of the literature is extraordinary and he provides good linking passages so that an overall picture of the interrelationships of these fields is built up.

Within the biological sciences Hinde includes comparative and experimental psychology whose interactions with ethology were often stormy. The personalities, as well as the scientific achievements, of people such as Schneirla. Lehrman and Beach were of great importance in determining the course of the interchanges and Hinde gives enough of the history to make us fully aware that science is a human activity. The influence of ethology in the human social sciences has also met with opposition. The contributions it can provide are, first, the comparative method, revealing the human species as one amongst many which may share certain attributes, and secondly a secure descriptive method for the early stages of behavioural analysis. Neither of these appealed to those social scientists who emphasized the uniqueness of the human species or who concentrated solely on the cognitive and perceptual aspects of human behaviour.

Such extreme positions are rarer now. The study of non-verbal communication. of the attachment between mothers and infants and of the effects of maternal separation have all very obviously gained from ethological techniques and concepts. Hinde himself has made valuable contributions in these areas and his sharp and revealing focus is clearest in these sections of the book. The critical edge is somewhat blunted when dealing with the biological approach to anthropology where some of the wilder shores of sociobiology are dwelt upon rather too gently, but all is brought back into balance in his concluding remarks.

This book will be of great value to students and teachers in other fields who want to broaden their ethological horizons. It also provides a liberal education in these other fields for more biologically based ethologists.

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An ode to adaptive transformation

Niles Eldredge

Darwinism Defended: A Guide to the Evolution Controversies. By Michael Ruse. Pp.376. ISBN 0-201-06273-9. (Addison Wesley:1982.) \$12.50, £8.40.

THINGS seem a bit frenetic in evolutionary biology these days. The casual observer might well think that the only theme imposing unity on this sphere of science is creationism: all evolutionary biologists agree that life has evolved, and all become willing allies in the political fight sparked by the recent successes of "scientific creationism" - which, however paltry its specific claims about natural history may be, asserts positively that life has not evolved. But beyond this simple and totally understandable closing of ranks, most observers see the current situation in evolutionary theory - where the object is to explain how, not if, life evolves - as bordering on total chaos. Seemingly, no two evolutionary biologists think alike these days, and we are less agreed upon things than we collectively were, say, a decade ago.

There is some substance to this view of disarray, but it has been exaggerated; we are more like a house divided than a potpourri of randomly diversified opinion. The basic problem is this:

FUSARIUM:

Diseases, Biology, & Taxonomy

ed by P E Nelson, T A Toussoun, & R J Cook

This volume brings together what is currently known about the pathology, ecology, genetics, cytology, physiology, biology, and taxonomy of this important fungus genus.

Penn State University Press, April, 560 pp, 7" x 10", 65 illus, £27 65

NATURAL HERITAGE

Classification, Inventory & Information

by Albert E Radford, Deborah K Otte, Lee J Otte, Jimmy R Massey, Paul Whitson, & contributors

The ultimate goals of this study are to foster the conservation of natural heritage, to advance field training in ecology, systematics, and evolution, and to determine site-population relationships for species of special concern.

University of North Carolina Press, 485 pp, 8½ x 11", maps & figures, £17 50

> AUPG 1 Gower Street London WC1

molecular and developmental biologists, systematists and palaeontologists all think their data have something to do with evolution. This is reasonable on the face of it because, whatever else it might be, evolution involves the modification of DNA sequences, development patterns, and species and higher taxa. And the latest news from Precambrian palaeontology tells us that the process has been going on for a minimum of three-and-a-half billion years.

On the other hand we have an evolutionary theory - the "modern synthesis" - that insists that all such evolutionary phenomena are entailed by the neo-Darwinian paradigm: organisms within populations vary amongst themselves, offspring tend to resemble their parents, and because in each generation more are usually produced than can possibly survive and contribute genes to the next generation, we get deterministic, albeit statistical, patterns of change in genetic representation in succeeding generations. This, of course, is natural selection. We now feel we know more than Darwin did about why organisms resemble their parents, and what the proximate and ultimate sources of variation might be. This understanding, plus Darwin's basic arguments, combine to yield the neo-Darwinian paradigm. No less an authority than Ernst Mayr (for example in his prologue to The Evolutionary Synthesis: Harvard University Press. 1980), has written that the "modern synthesis" is simply this paradigm of adaptive transformation plus a single additional concept: all evolutionary phenomena, of whatever scale - from molecules to phyla — are explicable in the familiar terms of population genetics. In the apt (if by now rather trite) phrase, the theory is both necessary and sufficient to explain all known aspects of evolutionary history. However oversimplified such a characterization of the synthesis may be, it fits to a T the viewpoint espoused by Michael Ruse in his new book, Darwinism Defended: A Guide to the Evolution Controversies.

The simple statements of the synthesis have long been taken as very general: all sorts of phenomena which hitherto seemed to cry out for separate explanations could all now be explained under one general theory of adaptive change. And, after all, if life has had a single history, it is indeed logical to assume that there must be a single, integrated theory of the process that engendered that single history. But now the synthesis is beginning to look exceedingly narrow. For consider the effect the synthesis has had: contrary to Ruse's enthusiasm for natural selection as a "research tool", the real effect has been the relegation of molecular and

developmental biology, systematics and palaeontology to the role of mere reportage. Evolutionarily-inclined members of these diverse disciplines have been told, in effect, to sit back, relax and simply recount the results of the evolutionary process — and leave it to the population geneticists to explain how it all happened. From time to time, members of one or more of these various satellite evolutionary disciplines have objected, insisting once again that their data - their molecules and fossils - must have something more direct to say about the validity of this or that notion about how life really does evolve. Now their voices are becoming more strident.

Michael Ruse is aware of this state of affairs. Hence the book, which has two titles, accurately conveys his priorities. First, it is a stout defence of what Ruse calls Darwinism. Secondly, and only through the glasses of a truly arch-conservative, the book touches upon virtually all the major issues of which I am aware. I call Ruse an arch-conservative advisedly: to this staunch defensor fidei, genetic drift is still a heresy. Anything smacking of the stochastic, or even of "neutrality", has him shaking his head and wagging his finger in a trice.

Darwinism, to Ruse, seems to be adaptation through natural selection. I say "seems" simply because his enthusiasm sometimes gets the better of him, and Darwinism in places comes close to meaning simply "evolution". Ruse knows better, of course, and generally makes the distinction between the notion that all living things are interrelated, and specific ideas (of which Darwinism is one) about how life has evolved, satisfactorily explicit.

Ruse has structured his book rather well. There are five main sections; the first three deal with his view of Darwinism yesterday, today and tomorrow. The first section gives us a brisk account of the man and his main works — with emphasis on Ruse's analysis of the Origin as a three-part essay. "Darwinism Today" tells us of the "coming of Mendelian genetics", what it is and what it means. Part 3, "Darwinism Tomorrow", is an eclectic grab-bag of topics, ranging from the "origin of life" to the "challenge from palaeontology". By this stage the reader will have no difficulty in predicting Ruse's line of argument. Those areas of contemporary biology stressing adaptive explanations through selection are "good", while he takes a dim view of disciplines and ideas which seem, somehow, to diminish the central role of adaptation. Thus sociobiology is "good", but punctuated equilibrium theory has the malodorous air of yesterday's half-eaten fish and chips. Part 4, "Darwin and Humankind", deals with the notion that Homo sapiens has had an evolutionary history, proclaims human sociobiology as on the side of the angels (if only metaphorically) and says that one should not adduce nasty ethics of the dog-eat-dog

variety just because sociobiology can explain so much of our behavioural history. I found this section, and the twochapter, fifth and final section on creationism, unprepossessing.

The guts of the book lie, appropriately enough, in its middle, in parts two and three. Here Ruse tells us what evolutionary biology really is, and should be, all about. I find it a dismal picture. The approach Ruse advocates is pure storytelling — or, as he himself puts it, continued indulgence in evolutionary biology's "favorite parlor game": concocting plausible scenarios of how the elephant got its trunk, the rhino its wrinkled skin and the giraffe its long neck. To Ruse, we know the mechanism, and it's all just a bunch of clever games applying this mechanism of genetic change to explain literally all manner of evolutionary phenomena.

Most critics of contemporary evolutionary theory, myself included. would agree that natural selection is a deterministic mechanism accounting for generation-by-generation change in gene frequencies within populations. It is even testable, given the appropriate data -which involve gene frequencies and generations. But reducing, say, ammonite evolution to a just-so story with natural selection as the hero is, as Ruse says, nothing but a parlour game. The difference between us is that Ruse thinks this state of affairs is just fine. I think parlour games are fine too — but I still cherish the fantasy that I am doing, or at least am trying to do, science when I'm practising my profession.

Ruse uses analogy and circumstantial evidence as his cornerstone criteria for establishing the validity of scientific ideas. Claiming Darwin used Herschel and Whewell as models, and that analogy and inference were what made science "good" in the 1850s, Ruse (as he does so much throughout the book) in effect argues that what was good enough for Darwin should suffice for us as well. There is little here of the spirit of enquiry. We are not enjoined, for example, to hold our notions lightly, to walk humbly before Mother Nature, ready to modify our schemes should the evidence of our senses not rhyme with our fondest notions.

Nowhere in this single-minded tract is Ruse's dedication to the almighty principle of natural selection better displayed than in his little section on trilobite vision. The mathematically "perfect" shape of phacopid and dalmanitid lenses is, to Ruse, exquisite evidence of the power of natural selection. I confess I cannot fathom the difference between Ruse's argument and the older creationist argument from design: see this organ system; observe its intricacy! Only (God, natural selection) could have fashioned such a marvellous organic machine! There is a difference, of course: God, as a supernatural being, does not belong in science, whereas natural selection patently does. But used in this inappropriate fashion, natural selection

becomes a mere substitute for the Creator. It tells us nothing, really, about trilobite eyes or anything specific or meaningful about how they came into existence.

In short, Ruse is a reductionist - all large-scale evolutionary phenomena are readily explicable in terms of population genetics. But there is more than mere reductionism here: he also dismisses molecular biology, simply because population genetics revealed the contents of Darwin's "black box" of heredity, and molecular biology, Ruse avers, has not changed that one whit. All remains secure. That there are patterns of organization with historical implications emerging from molecular and developmental biology that seem to require their own mechanisms in addition to those of population genetics is missing in Ruse's world view. Indeed, in his reflexively negative reaction to the winds of change, Ruse fails to see that the newer arguments are nearly all additive: natural selection explains changes in gene content and frequency within populations, but more than populations are involved in evolution. Another example: Ruse doesn't even bother to mention the idea that species are individuals - the ontological claim underlying the view that macroevolution

may be something more than just scaled-up microevolution.

Darwin and his ideas need no defence. I agree with Ruse: Darwin was a good scientist. In that spirit I would rather imagine Darwin enjoying the spectacle of biologists vigorously debating his, and descendant, notions over a century after the Origin. Ruse, appropriating Darwin's shade as his alone, seems to think that to question some of Darwin's notions is somehow to impugn the man. So he springs to the defence. It is a clumsy defence, one that certainly does Darwin no credit. As a statement of the nature and effective extent of neo-Darwinism, it is nowhere as good as, say, the September 1978 issue of Scientific American, or any of a number of recent texts. As a book aimed at a general audience, it projects a jolly Alice in Wonderland sort of picture that, I fear, will not do overly much for our collective image in the long run. Darwinism, indeed, the entire field of contemporary evolutionary biology, deserves far better.

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welcome. Following hard on the heels of

his influential scientific text Macro-

A new paradigm for evolutionary change?

R.D. Martin

The New Evolutionary Timetable: Fossils, Genes and the Origin of Species. By S.M. Stanley. Pp.222. US ISBN 0-465-05013-1; UK ISBN 0-633-7022-8. (Basic Books/Harper & Row: 1982.) \$16.75, £9.50.

IT is, perhaps, appropriate that the centenary of Charles Darwin's death should be approximately marked by renewed controversy over the process of evolutionary change. The latest debate concerns one of the most fundamental features of Darwin's theory of evolution by natural selection - gradual change within individual species populations over time. An alternative interpretation, stated with particular clarity by Eldredge and Gould in a seminal paper published in 19721, is that most evolutionary change occurs when a new species develops (during a "speciation event") and that there is little change subsequent to establishment of a species.

In some circles, at least, replacement of the Darwinian concept of "gradualism" by the new model of evolution by "punctuation" has been hailed as a fully-fledged scientific revolution (a Kuhnian paradigm change). To date, however, the debate has raged largely among academics, and for this reason Steven Stanley's The New Evolutionary Timetable is both timely and

evolution; Pattern and Process (W.H. Freeman, 1979) this new book attempts to present the case in terms understandable to the non-specialist. Stanley achieves his aim with considerable success, carrying the reader along with a style that is at once lively and informative. Penetrating insights abound as the arguments are presented, and frequent touches of wry humour add a special touch. (Any modern biologist struggling for a command of the literature will surely be relieved to learn that Darwin actually possessed an uncut copy of Mendel's monograph on hereditary mechanisms!) Certainly, a reading of this book leaves one with plenty of food for thought, regardless of the correctness of Stanley's own conclusions. This is a particularly productive time for the remodelling of evolutionary theory, and The New Evolutionary Timetable conveys the excitement felt by many biologists involved.

Underlying the concept of punctuated evolution is an undeniable fact derived from recent palaeontological studies: numerous fossil species have been found which exhibit a remarkable degree of stability ("morphological stasis"), over long periods of geological time. It must at once be added that such statis can only be recorded for certain body parts (molar teeth of mammals or shells of molluses, for

¹Eldredge, N. & Gould, S J in *Models in Paleobiology* (ed Schopf, T J.M), 82-115 (Freeman & Cooper, 1972)

example) and it is reductionist to conclude that entire organisms remain unchanged over time. Differential evolution of individual body components (mosaic evolution) is a widely recognized phenomenon and there is, for instance, no indication of stasis at the level of the structural gene. Stanley makes little mention of the substantial literature on molecular evolution, which suggests a hiatus between rates of evolution of single proteins and those of whole organisms. It is all too easy to slip into equating fossilized skeletal parts with entire organisms, and this slip is a recurrent feature of the literature of punctuated evolution. Stanley, for example, asserts that Homo sapiens has undergone almost no bodily change since appearing in Europe some 40,000 years

The second aspect of the punctuational model is rapid evolution during speciation, which is generally assumed to occur in relatively or very small, peripheral populations. Such rapid evolution must, however, be *inferred* from the fossil record and involves the assumption that the fossil record is sufficiently complete to document this process unequivocally. In a few special cases this may be true, but the fossil record as a whole is pitifully inadequate — there is, for example, only one major fossil site recording the occurrence of mammals in the entire African continent for the first 35 million years of the Tertiary. We really

need to have estimates of the relationship between the likely catchment areas of relevant fossil sites and the geographical ranges of equivalent modern species; in the absence of such estimates a reasonable guess is that fossil sampling is in fact extremely patchy.

Nevertheless, the picture now emerging from the fossil record is unsettling, and our ideas of evolutionary processes must undoubtedly progress to account for longterm stasis, at least in individual body parts, in species populations. The question is whether such progress requires no more than healthy readjustment of basic theory or whether the changes which occur during speciation dictate recognition of a radically new process ("quantum speciation" or "macroevolution"). Stanley is convinced that the latter is the case. Perhaps understandably, he does not fix upon a single basic mechanism of "macroevolution". though he does somewhat vaguely implicate chromosomal reshuffling. Everything really hangs on the rapidity with which speciation takes place. Stanley states that he is prepared to envisage thousands of generations as the time-scale for "quantum speciation", and this could well be accommodated within a traditional model of evolutionary change. Stanley feels that the fossil evidence for human evolution provides us with one of the best illustrations of punctuation, yet "thousands of generations" could occupy a considerable proportion of the average geological duration of the hominid species currently recognized. In any case, the situation is far more complex than Stanley suggests, notably in the existence of intermediate forms (see Cronin et al., Nature 292, 113-122). Stanley himself is somewhat unclear about the actual limits of his model as well. He suggests (p.157) that human races ("subspecies") may have arisen by punctuated evolution. Quite apart from the fact that here he is treading on extremely contentious ground in sociopolitical terms, it is difficult to see what 'quantum'' differences might define the human races in any context relevant to punctuated evolution.

In the absence of a clear model of the mechanism of "quantum speciation", the case for a radically new paradigm remains somewhat unconvincing, as was the case with continental drift prior to the recognition of sea-floor spreading. Stanley devotes a whole chapter to demonstrating that his doubts about conventional theory do not open the door to creationism; but without a clear concept of the mechanism involved in "punctuated evolution" he is unlikely to convince any creationist! Stanley is right to alert the general reader to the surprising new findings of palaeontologists, but he really goes much farther than the present evidence permits.

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Two sides of Darwin

A.J. Cain

Darwin. By Jonathan Howard. Pp.101. Hbk ISBN 0-19-287557-4; pbk ISBN 0-19-287556-6. (Oxford University Press: 1982.) Hbk £5.50; pbk £1.25. To be published in the USA later this year. Darwin. By Wilma George. Pp.160. ISBN 0-00-636502-7. (Fontana: 1982.) £1.75.

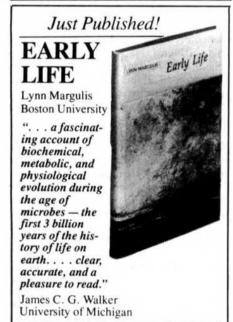
It is a sign of Darwin's relevance that Jonathan Howard's book is in the OUP Past Masters series, Wilma George's in the Fontana Modern Masters. They are nearly identical in their rather nasty format (dirtycoloured paper, undistinguished type-face and over-gorgeous covers, rather like the trash-novels on a railway bookstall), but do not be put off by first appearances - their intellectual content is very good, and both are well worth the money. Both are written by practising zoologists - a refreshing change from so many books on Darwin but zoologists of very different interests. Jonathan Howard is a Principal Scientific Officer at the ARC Institute of Animal Physiology at Babraham, Wilma George is a University Lecturer in the Department of Zoology, Oxford. There is also a useful difference of sex; some of Wilma George's comments on the acceptability of Darwin's theories of sexual selection and male aggression to Victorian men are particularly enlightening.

Jonathan Howard remarks in his preface that

With the centenary of Darwin's death comes a widespread mood of scepticism and unease about the validity and significance of Darwin's contribution to knowledge. The time is certainly right to try to describe Darwin's scientific work briefly and in plain language.

While fully recognizing the importance of Darwin's work for biology, he says "I hope the book is fair in pointing out aspects of Darwin's thinking which lack consistency or have failed to stand up to critical scrutiny". And he rightly emphasizes that whatever others have made of Darwinism in philosophy or sociology, and however true it is that "human life and human society are to a certain extent biological issues", it is Darwin's contribution to biology on which he must stand or fall. "Darwinian philosophy of Darwinian society are post-hoc constructs that had no place in Darwin's thought".

The chapters take us successively through Darwin's life; the foundations of Darwinism; natural selection and the origin of species; the evidence for evolution by natural selection; sex, variation and heredity; man; perfection and progress; Darwinism and ideology; and Darwin as a scientist — an evaluation. The style is mostly good and the exposition clear. Just occasionally it would have benefited from critical reading — for example I can make no sense out of Howard's comment on



"Great resource and beginning reading for the non-specialist who wants to learn about the evolution of early life."

> Kenneth H. Nealson Scripps Institution of Oceanography

Feb. 1982, 176 pp., 62 illus, Glossary, \$9.95 (paper), \$16.50 (case), **Science Books International**, 51 Sleeper Street, Boston, Mass, 02210. Write to Jane Wescott for purchase or examination copy, or call (617) 426-2224.

Circle No.03 on Reader Enquiry Card.

Darwin's rejection of Christianity (p.9):

Darwin reserved this uncompromising expression of his religious views for the private autobiography that he wrote for his family. Had he not done so it is inconceivable that T.H. Huxley could have abandoned his war against hypocrisy and helped to carry Darwin's coffin into Westminster Abbey in 1882.

It is inconceivable that Huxley did not know Darwin's views on religion — Darwin was not reticent about them to correspondents — and that he should not have applauded them. Where was the hypocrisy?

Howard's book is essentially a careful exposition and critique of Darwin's arguments in the *Origin* (and in the *Descent of Man* for the evolution of man). It is in the structure of the arguments that he is principally interested. Of course he does not neglect the factual evidence, but produces usually only the minimum of it—for example his comments on the cogency of geological, geographical, taxonomic and embryological information in establishing that evolution had occurred (pp.37–49). Similarly, in summarizing the modern position on natural selection he merely remarks (p.87),

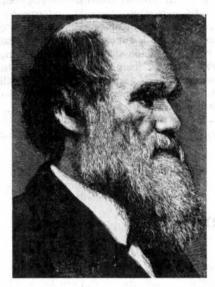
Natural selection and the evolution of natural populations have been observed directly, even in man. There is now no doubt that natural selection is a mechanism of evolutionary change. Whether it is the exclusive mechanism of evolutionary change is still occasionally debated.

And he accepts Darwin's own belief that he had spent too much time on barnacles (p.5) without analysing what he had got from them.

Howard's exposition of ideas and their inter-relatedness is much more full, and he

seems especially interested in predictability and unpredictability and their role in the evolutionary debate. He remarks (p.24):

If mutual competition between organisms was a more important selective influence than mere climatic or geographic change, then the direction of evolution of a species or of an interacting group of species, became effectively impossible to determine.



And, after a supporting quotation from the *Origin*.

It is important to recognise this instance for what it is, namely a hypothetical scenario for fluctuations in frequency of different kinds of organisms that are all subtly related to each other, initiated by introducing into a dynamic equilibrium a single small change....

Here is the physiologist pre-adapted for seizing the complexities of a dynamic system and comprehending the modes (and pitfalls) of its analysis — a valuable approach.

Wilma George's book, written in a particularly attractive and lively style, inevitably covers much of the same ground but is effectively complementary to Howard's in its mode of treatment. She has travelled extensively, realizing the facts of natural history, adaptation and biogeography at first hand - an equally valuable approach. She uses all of Darwin's works very effectively, showing how his interests grew on a wide front, and provides the best short account that I know of just why he was so interested in barnacles, what he got from them and how the ideas acquired led on to later interests (polymorphism, sexuality, insectivorous plants and the fertilization of orchids, for example). Her insight into the development of his ideas on coral reefs in relation to other geological ideas is particularly clearly expressed, and she relates Darwin most effectively to other workers before, at the time, and after. She gives a more concrete exposition of subsequent work in population genetics, natural selection and speciation, than does Howard.

Either book can be read with pleasure by itself; the two combined make a first-class introduction to the subject. Both authors make suggestions for further reading, to which should be added Dorothy Nelkin's spine-chiller Science Textbook Controversies and the Politics of Equal Time, published by MIT Press in 1977. Both glance at the present situation in evolutionary thought, and both expatiate on the forces of unreason, but neither, it seems to me, appreciates just how powerful and dangerous they are. Nelkin's book is a necessary addition.

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One foot in sea, and one on shore . . .

D.R. Newth

The Aquatic Ape: A Theory of Human Evolution. By Elaine Morgan. Pp.168. ISBN 0-285-62509-8. (Souvenir Press, London: 1982.) £7.95. To be published in the USA later this year by Stein & Day.

Sigh no more, ladies, sigh no more, Men were deceivers ever; One foot in sea, and one on shore, To one thing constant never.

ONE of the few changes made by Darwin in preparing the second edition of the *Origin of Species* was the exclusion, in obedience to Lyell, of any reference to the "secondary whale". The evolution of aquatic mammals still presents troubling problems, but at least, for the most part, those mammals taking to water do not appear to have had second thoughts. It was, therefore, brave of Alister Hardy to

suggest, nearly a quarter of a century ago, that mankind had passed through a semiaquatic phase and that some of our less easily explained biological features were its legacy.

Hardy laid stress upon our relative hairlessness - and upon the orientation of the hairs that we do possess, upon bipedalism, upon subcutaneous fat, upon our ability to swim, and upon the form and sensitivity of the human hand. All seemed to make sense as adaptations to littoral life, less aquatic than that of present-day otters, but one sufficiently dependent upon shallow waters to make the transition to bipedal locomotion easier to envisage in view of the buoyancy enjoyed by the wading anthropoid. Although Hardy did not attract much support for his idea he was content to wait for the matter to be decided by the fossil precursors of the australopithecines when their discovery should fill the late Miocene-early Pliocene gap in the story.

In bringing us, with Sir Alister's blessing, an extended statement of the case for his ideas, Mrs Morgan adds arguments of her own. She sees in face-to-face copulation an important behaviour pattern shared by mankind and many aquatic mammals, but not seen in infra-human primates. Emotional weeping is another such behaviour. She also believes that speech as a mode of communication may have evolved as the limitations of smell and sight in an aquatic environment made themselves felt. In this, and in the suggestion that underwater childbirth may once have been the norm, she seems to envisage a more completely aquatic phase than Hardy originally did.

The additional arguments do not, I think, greatly strengthen the case. Mrs Morgan herself sees face-to-face copulation in mankind as a consequence of bipedalism and hence, at most, as a secondary consequence of an aquatic phase. On the shedding of tears the position is not simple. While seals and sea

otters apparently weep for emotional reasons as do we, so too do Indian elephants. Elephants like whales have, however, reduced their lachrymal gland and increased the Harderian gland contribution to tears. Mrs Morgan is willing to accept elephants as fellow travellers to the water and back, on this and other grounds, but until we are more confident about the functions of copious tear production it seems best to be cautious.

However the fossil record will doubtless have the final say, and it is becoming yearly more complete. Mrs Morgan re-publishes the suggestion of another supporter of the Hardy hypothesis, L.P. La Lumiere. He believes that the aquatic phase was experienced during early Pliocene times on the coast of the Red Sea. At a time of presumed deforestation, changes in land

and water levels providentially provided an island refuge. La Lumiere offers precise predictions of where the missing links will be found.

This book is written for a lay public and is too brief, and too superficial, to satisfy a serious critic. The bibliography, for example, fails to include many of the authorities referred to in the text. The book is also marred by occasional carelessness—I had to read one passage several times to extract from it a meaning other than that women and sirenians were alone in having two pectoral mammary glands. But the writing is lively and enthusiastic and it will surely engage the interest of those for whom it was written.

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A cycle ride to Stockholm

M.F. Perutz

Reminiscences and Reflections. By Hans Krebs in collaboration with Anne Martin. Pp.250. ISBN 0-19-854702-1. (Oxford University Press: 1982.) £12.50, \$19.95.

On 14 December 1932 the dean of the medical faculty of the University of Freiburg, Professor E. Rehn, reported to the Ministry of Education,

As an assistant physician Dr Krebs has shown not only outstanding scientific ability, but also unusual human qualities. His . . . paper on the synthesis of urea in the animal body . . . will be regarded as one of the classics of medical research

Four months later, shortly after Hitler seized power, this same dean sent him a "Notification of Immediate Removal from Office", obediently implementing the Minister's orders against members of the Jewish race.

Krebs kept several such letters and also various press cuttings from those days and had them reproduced as facsimiles in his book. There is the "Manifesto Against the UN-German Spirit" published by the Freiburg Student Union and posted in the University: "Our most dangerous adversary is the Jew and he who serves him. The Jew can think only as a Jew. If he writes German he lies. The German who writes German, but thinks un-German is a traitor . . . ". The Rector of the University, von Möllendorf, ordered the posters to be removed, whereupon the Minister replaced him by the existentialist philosopher Martin Heidegger who endorsed them and proclaimed, "German students! . . . Your existence must not be ruled by learned axioms and ideas Only the Fuhrer himself constitutes today's and tomorrow's reality and law. Daily and hourly must you fortify your faithful

obedience ...'. Most of Krebs's colleagues acquiesced, but one Dr Arthur Jores dared send his and Krebs's former Jewish chief, by then in New York, a reprint with a personal dedication. The envelope was opened by a Nazi colleague who denounced him. Jores was dismissed from his post, publicly branded as an enemy of his country and, in due course, imprisoned.

Despite these harrowing experiences and the extermination of 20 of his relatives Krebs does not follow the example of Einstein who refused ever to set foot in Germany again, but declares in the book that "to be anti-German seems to me just as bad as being anti-semitic". After the War he therefore persuaded the Biochemical Society to re-establish contact with Germany by inviting several known anti-Nazis to the First International Congress of Biochemistry at Cambridge.

Krebs's discovery of the ornithine cycle of urea synthesis had caught the eye of F. Gowland Hopkins, then Professor of Biochemistry at Cambridge and President of the Royal Society. When he heard of Krebs's dismissal, he immediately invited him to Cambridge. The Rockefeller Foundation which had already supported Krebs's research at Freiburg, provided the money, and continued to support his research for the next 30 years. Krebs was deeply touched by the warmth of his reception at the Biochemistry Department at Cambridge and by the generous hospitality and friendliness of English people generally. His book is permeated by affection for England, which he epitomizes by Carl Zuckmayer's, another refugee's, saying that "home is not where a man is born but where he wants to die".

How Prussian Krebs remained all the same! He writes,

... I expected a lot from my associates — hard disciplined work and the ability to accept my criticisms . . . I have tried to be fair; honest and helpful, and not to demand more of others than I do of myself We criticised each other ruthlessly — we knew it was done honestly, in good faith and in a spirit of helpfulness.

Few Englishmen would write such solemn stuff about themselves. I cannot imagine Francis Crick declaring in his memoirs that he demolished my cherished 1949 model of haemoglobin, "honestly, in good faith and in a spirit of helpfulness", rather than admitting to a certain mischievous satisfaction at the dastardly deed.

For people who complain that money for research has become hard to come by. Krebs's early career makes salutary reading. His father, a doctor in Hildesheim, had to support him throughout his medical studies. After graduating Krebs wanted to do research. but paid research posts were rare, never advertised and obtainable only by what used to be known in my native Vienna as Protektion, which signified a mix of sycophancy, nepotism and the old boy network. Eventually Krebs found an unpaid post at the Third Medical Clinic in Berlin where another biochemist who later made his name, Bruno Mendel, became one of his colleagues. One night the Mendels were invited to the Einsteins when Otto Warburg was among the guests. When Warburg told Mendel that he was looking for a collaborator, Mendel recommended Krebs and also raised the money privately to pay him a modest salary. Krebs regards his four years with Warburg as formative for his career and expresses the greatest admiration for Warburg's scientific genius, regardless of his despotic, egotistical and sometimes malicious behaviour, his lack of confidence in Krebs's own talent and his refusal to help him find a university post where Krebs would have to attach himself "to some old ass of a professor". Only in medicine would Krebs be able to make a living, Warburg told him. Warburg demanded of his staff that they work punctually from eight to six, six days a week, a precept which Krebs himself seems to have followed for much of his life. At 11 o'clock on the Monday morning after reading of Warburg's Draconian regime, I walked into my own biochemistry laboratory to find that only one of my three collaborators had arrived yet. "The other two were still working when I left at midnight", the first reported. This convinced me again that the free coming and going of Cambridge is more conducive to dedicated research than the iron discipline of old Berlin.

Krebs made his first great discovery, the ornithine cycle of urea synthesis, in 1931 and 1932. He used the tissue slice and manometric techniques which Warburg had taught him; another decisive factor was his own invention of the Krebs-Ringer

solution. I found it exciting to read of his groping in the dark, methodically testing all conceivable intermediates until he discovered that "one molecule of ornithine could bring about the formation of more than twenty molecules of urea, provided that ammonia was present". From that moment the tracing of the other intermediates followed logical steps. It was the first biological process in which the intermediates were found to play a purely catalytic role. Krebs made this fundamental discovery while in charge of a medical ward with over 40 beds, which makes his feat even more remarkable.

Krebs's unravelling of the citric acid cycle in 1937 was to win him even greater fame, but his letter to *Nature* announcing it was rejected by the editor, Sir Richard Gregory, who at that time took it upon himself to judge the scientific worth of most of the communications sent to him. (It is not known whether Krebs's paper was refereed, but that seems unlikely — Ed.)

Objections were raised at first against both the cycles. Those against the ornithine cycle were later found to be based either on wrong experiments or incorrect interpretation, while those against the precise chemistry of the citric acid cycle seemed fundamental. Biochemists argued that radioactively labelled CO₂ introduced into the cycle should become randomly distributed among the two carboxyl groups of α-ketoglutaric acid, an intermediate two steps after citric acid, because an enzyme would not be able to distinguish between the two symmetry-related carboxyl groups of citric acid. In fact, only the carboxyl nearest to the keto group of a-ketoglutaric acid was found to be labelled; hence, it was concluded, citric acid could not be an intermediate in the Krebs cycle. That was in 1941. I would have been desperate if an apparently valid objection had been made to my most fundamental discovery in which I could detect no flaw, but Krebs writes as though it had never cost him any sleep. Was he really so placid that he did not continuously turn over in his mind all conceivable explanations of the paradox, or did the sunshine of his later glory dissolve the memory of the seven clouded years that were to elapse before A. Ogston, in a brief and classic note to Nature, pointed out the fallacy in the objection: a symmetric molecule attaching itself to an enzyme at three points may give rise to only one of two possible asymmetrical reaction products. This was the birth of the concept of prochirality.

The glory was first heralded in October 1952 by eager journalists who told Krebs that he would shortly be awarded the Nobel Prize, but the rumours proved false and S.W. Waksman received it instead. Krebs relates proudly that the rumours left him and his wife unruffled. But did they really? Nine years later similar rumours about John Kendrew and me were floating

Scherzo metabolico cantabile

Thomas A. Scott

The Biochemists' Songbook. By Harold Baum. Pp.62. Pbk ISBN 0-08-027370-X. (Pergamon: 1982.) £2.45, \$4.95.

How refreshing, in the 1980s, to find a professor of biochemistry writing, not a solemn research text, but humorous biochemical verses. Thirteen important topics of biochemistry have been rendered by Professor Baum into light-hearted songs and set to appropriate popular tunes with piano accompaniment. The biochemical accuracy of the outrageous doggerel can be checked at a glance, because each song appears with the conventional scheme or account.

According to the author, all the songs were written while travelling on the top deck of a No. 22 bus; this I do not believe. He also points out that they are intended for communal singing, ideally with a blood alcohol level of 35 mg per cent; this I do believe. Some of the scansions are tortuous and not always immediately obvious. While the alcohol will not make them any easier, it will stop you worrying whether you get them right.

Perhaps the greatest challenge is in the first verse of "Protein Biosynthesis":

Introns and exons, changes post-transcriptional, and all Glycosylations, don't alter such basics at all

to the tune of My Bonny Lies Over the Ocean. In contrast other compositions have a reassuring and earthy quality, for instance:

If you gobble tagliatelli, Chicken soup with vermicelli, You'll acquire a sagging belly —

are the opening lines of "Fatty Acid Biosynthesis" set to Men of Harlech.

Surprisingly, the author has failed to exploit the rousing coda of Macnamara's Band in "The Pentose Phosphate Shunt". Admittedly, this loud and rasping interlude is normally left to the trombones, but it is tailor-made for Professor Baum's particular style of ethanolic chorus; in the second edition perhaps? Also, these days, when so many bar pianists are over forty, and all the young wags play the guitar, this already excellent songbook might be made more usable by the younger generation by the inclusion of harmony frets or chord names for each tune.

The Biochemists' Songbook is dedicated jointly to Sir Hans Krebs on his eightieth birthday and to the author's wife. Sir Hans has also contributed a foreword, and there is a certain poignancy in the publication of the Songbook so shortly after his death. His cycle is set to Waltzing Matilda, and this year the International Congress of Biochemistry will be held in Australia, where it is no novelty, indeed it is a long established tradition, to sing this tune communally and with a blood alcohol level rather in excess of that recommended by Professor Baum.

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around our laboratory. We doubted them until my secretary rushed in, flourishing two telegrams, one addressed to Kendrew and the other to me. This was it. When we had eagerly torn them open we found them to be from the Pontifical Academy in Rome, enquiring how many reprints we wanted of the papers we had read there the previous autumn. We also pretended to a stoic calm. Unruffled or not, Krebs did receive the Prize the following year.

After nearly 20 happy years and the founding of a flourishing school of biochemistry at Sheffield, Krebs became

Professor of Biochemistry at Oxford and stayed there until his death last November. I was surprised by his statement that Oxford had remained in the forefront of learning for 600 years. Had he never read Edward Gibbons's description of its decline into sloth in the eighteenth century when dons could not even be bothered to teach their students? The picture of Oxford University in Krebs's own time conjured up in the book is of a citadel of unjust privileges jealously guarded by the Party members. Krebs got himself elected to the Central Committee by the under-

privileged Solidarity of Active Researchers, but all his brave attempts to reform The System were defeated by the strength and cunning of the Old Guard, who even went so far as to refuse money offered by the University Grants Committee for the creation of additional science professorships, lest this should lead to the appointment of more Dissidents and Enemies of the Party like Krebs. Despite these rebuffs, Krebs describes his life at Oxford as a happy and successful one. Asked once for a guiding motto, Krebs replied "Never put off till tomorrow what you can do today", which sounds like one of those recipes for virtue Victorian children were made to embroider and hang over their beds. The King of France says it less prosaically in All's Well that Ends.

Let's take the instant by the forward top; For we are old, and on our quick'st decrees, The inaudible and noiseless foot of time Steals ere we can effect them.

Krebs emerges from this book as a dynamic scientist and an engaging, warm-hearted individual utterly devoted to his research and teaching. He quotes Noel Coward's saying that "work is fun, there is no fun like work". I agree.

M.F. Perutz is a Member of the Medical Research Council Laboratory of Molecular Biology, Cambridge. With John Kendrew he was winner of the Nobel Prize for Chemistry in 1962.



AN ENUMERATION OF THE FLOWERING PLANTS OF NEPAL

Vol.1 Gymnospermae & Monocotyledones. H. Hara, W.T. Stearn & L.H.J. Williams. June 1978. **£22.50**

Vol.2 Dicotyledones I. H. Hara & L.H.J. Williams.

June 1979.

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Vol.3 Dicotyledones II. H. Hara, A.O Chater & L.H.J. Williams. May 1982. £32.00

The publication of the third volume completes this joint project of the British Museum (Natural History) and the University of Tokyo. It is based on the extensive botanical exploration of Nepal by British and Japanese scientists during the last 25 years.

Publications Sales, British Museum (Natural History), Cromwell Road, London SW7 5BD

Professors, parasites and public health

Anthony C. Allison

New Guinea Tapeworms and Jewish Grandmothers: Tales of Parasites and People. By Robert S. Desowitz. Pp.224. ISBN 0-393-01474-6. (W.W. Norton:1981.) \$12.95, £8.75.

DURING the period following the Second World War, a few pioneers of a particular type flourished. They were motivated by curiosity about medical problems in primitive societies, the people themselves and how social organization affects health. In an era of liberal funding they were able to spend a great deal of time travelling to New Guinea, South-East Asia, the Australian outback, East and West Africa and other appealing places, collecting thousands of samples of blood, brains and other materials. For some time they were regarded as amusing, if somewhat irresponsible, amateurs, who should settle down and do serious research in Bethesda or London. But they enjoyed themselves, satisfied their curiosity and carried out research of real quality, sometimes solving problems that had for decades defied frontal attack in the United States and Western Europe.

Carleton Gaidusek transmitted kuru, a degenerative disease of the central nervous system, from cannibals of the New Guinea highlands to chimpanzees. His energy and vision played a large part in development of the concept of slow viruses. Baruch Blumberg found that certain sera of North Americans who had received blood transfusions precipitated a component in the sera of some Australian aborigines. Out of this seemingly trivial observation came the identification of hepatitis B virus and the development of a test that has nearly eliminated post-transfusion hepatitis in the United States and Western Europe. Both of these developments won Nobel prizes. Following Denis Burkitt's observations on the prevalence of a particular type of lymphoma in Uganda and other East African countries, Tony Epstein identified in the lymphoma cells the herpesvirus that bears his name. This was found to transform human lymphocytes, to cause infectious mononucleosis and to be associated with a type of cancer of the nasopharynx common in parts of East Africa and China. It is the first likely candidate for a human cancer-inducing virus. The identification of the single amino-acid substitution in sickle-cell haemoglobin, and the demonstration that this gene confers resistance to malaria, were notable contributions to molecular and population genetics.

Included in this group of individualists is Bob Desowitz. After an orthodox training in tropical medicine, he worked on sleeping sickness in Nigeria and on various parasitic diseases in Singapore. He then became Professor of Tropical Medicine in Hawaii, and has travelled as a consultant for the World Health Organization and national governments to New Guinea, Indonesia, Burma and other places. This is a life that many might envy, and its highlights are recounted in this book of essays. Although the title might seem flippant, the book is instructive and entertaining.

Desowitz has a clear and readable prose style. Most medical students and biologists are bored by the life cycles of parasites. It helps to know that the fish tapeworm Diphyllobothrium latum was introduced into the fish of the lake region of Minnesota and Wisconsin by Scandinavian fishermen and was not killed during the delicate cooking of gefilte fish by New York grandmothers. The book is packed with easily assimilated information about parasites. Desowitz is an amusing raconteur and enjoys the detective side of public health, such as finding that a common brain infection among the Ekari of New Guinea was cysticercosis (tapeworm disease) transmitted by pigs given to the people by President Suharto of Indonesia to sweeten the military occupation.

The two central themes of the book are, first, that man-made ecological-environmental changes have been responsible for perpetuating and intensifying the majority of infectious diseases; and, second, that ultimately effective control of these infections will follow widespread use of the time-honoured, sensible approach of environmental sanitation.

Each of these assertions is open to debate. It is true that building the Aswan high dam in Egypt increased the already high risk of developing schistosomiasis in Egypt, but that has to be balanced against the manifest advantages of building such a dam. Whenever such enterprises are contemplated, the environmental health implications should be considered by international experts convened by the World Health Organization and the Food and Agriculture Organization. Determined efforts should be made to prevent such health problems; this would be a useful way to use international aid funds, which are often squandered.

Does control of disease necessarily require the time-honoured, sensible approach of environmental sanitation? Nobody would argue against sanitation. But with labour and fossil fuel costs escalating, there is no realistic prospect of eliminating malaria from Africa by ditches and drains or tsetse flies by scrub clearance. Despite 30 years of the Chinese "People's War against the Snail" and Chairman Mao's poem "Farewell to the God of Plague", schistosomiasis still exists in China.

It is more realistic to employ whatever

weapons are at hand. Environmental sanitation should be used wherever it is feasible, and coupled with health education. Immunization too, has its place. Smallpox was eliminated by a vaccination campaign carried out with military determination, and immunization markedly reduced the incidence of poliomyelitis in industrialized countries. Chemoprophylaxis and chemotherapy are also useful. They greatly reduced the prevalence of yaws and have protected millions of people and domestic animals from parasites. As in the case of chemoprophylaxis against filariasis in

Samoa, quoted by Desowitz, knowledge of the social organization of the people helped.

The essays of Desowitz provoke these and many other thoughts. They are recommended to students of medicine, public health and biology, to administrators of international health programmes and to those who enjoy a good read about science and the world in which we live.

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The making of an archaeologist

Colin Renfrew

Mortimer Wheeler: Adventurer in Archaeology. By Jacquetta Hawkes. Pp.416. ISBN 0-297-78056-5. (Weidenfeld & Nicolson: 1982.) £10.95.

"TURNING out of Pall Mall, I was transfixed by the steely gaze of Mr. Augustus John. 'Hullo, Rikki,' he said; 'still digging?'. 'Hullo, Augustus,' I replied; 'still sketching?'. With these words Sir Mortimer Wheeler (Rik to his friends) began Still Digging, his vivacious autobiography, published by Michael Joseph in 1955. Its subtitle, 'Adventures in Archaeology', is recalled in that of the biography of Jacquetta Hawkes, now published six years after his death.

There is no more equivocal gift to the biographer than a really good autobiography. The freshness and energy of Still Digging make it lively reading today, and its very excellence offers Jacquetta Hawkes something of a challenge, to which she has risen conscientiously and above all sympathetically, making considerable effort to get behind the public persona and reveal the human being.

It is indeed for that persona that Wheeler is still most widely remembered. For those too young to have been viewers, it is difficult to convey the national impact of his charismatic role, back in the 1950s, in that remarkable television programme Animal, Vegetable, Mineral. Both Wheeler and the question master Glyn Daniel became household names — they were elected "TV Personality of the Year" in 1954 and 1955 respectively. This was haute vulgarisation, in Glyn Daniel's term, popularization at its best and with a serious purpose. It made archaeology both better known and more widely understood, laying the foundation

● The third edition of *Digging up Bones* by D.R. Brothwell, published by the British Museum (Natural History) and Oxford University Press, appeared earlier this year. Price in paperback is £8.95.

in Britain not only for more substantial subsequent television coverage (the Buried Treasure series, and then Chronicle) but also, it could be argued, for the rapid growth over the past 20 years of archaeology as a degree subject which is numerically strong in a number of universities. As the author stresses here, Wheeler was, in his early excavating days in the 1920s, a pioneer of what today might be called public relations. But of course it was very much more than this: Wheeler saw his discoveries in human, personal terms and he never lost-the gift of catching the imagination of the non-specialist. He saw, too, that if the ultimate aim of archaeology is to inform us about the human past, it is the duty of the academic not only to research but to communicate. As he wrote in his best book, Archaeology from the Earth (Oxford University Press, 1954): "In a simple, direct sense, archaeology is a science that must be lived, must be 'seasoned with humanity'. Dead archaeology is the driest dust that blows".

In his later years, as Secretary of the British Academy, Wheeler made substantial contributions to the development of archaeology in Britain and indeed to the standing of the humanities in general. The British Academy, a much more recent creation than the Royal Society, has even now not quite achieved the active leadership within its own field which its elder sister has among the sciences. That the two are nonetheless at least comparable owes much to Wheeler's leadership during his tenure as Secretary from 1949 to 1968, as Jacquetta Hawkes describes most effectively.

Undoubtedly, however, Wheeler's main claim to lasting fame rests with his work as a pioneer of archaeological field methods. His incisive and systematic approach to the problems of excavation was not entirely new — as he generously acknowledged in the preface to Archaeology from the Earth, many of the methods and principles which he used were derived "from those of the

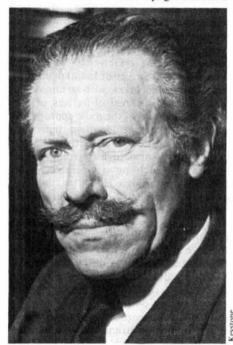
greatest of all archaeological excavators General Pitt Rivers". It was to those largely forgotten publications from the end of the last century that Wheeler turned, and with their aid developed systems of recording, with emphasis upon the interpretation and drawing of stratigraphic sections, which transformed excavation in Britain from a pastime into a discipline.

Wheeler's most personal contribution was an unfailing grasp of what he called the Tactics and Strategy of excavation. Those same qualities which produced a first-class soldier (he rose to the rank of Brigadier in the Second World War and fought at El Alamein) and highly effective administrator (Director General of the Archaeological Survey of India) made him a masterly field worker, with an unrivalled perception of the real objectives of the work in hand and of the most appropriate measures for their achievement. Indeed, it could be argued that with Wheeler excavation became to archaeology what experiment is to the physical sciences — the opportunity through clear thinking and careful planning to try out new ideas and to test old ones. Of course British archaeology overseas had other pioneers of field method besides Wheeler - Sir Flinders Petrie, as Wheeler himself acknowledged, or Sir Leonard Woolley or Sir Max Mallowan. But Wheeler was the unflagging advocate of sound field method as the essential basis of further work, and his teaching in the years after the First World. War promoted in Britain an awareness of stratigraphic principles which persists today and is ultimately sounder and more productive than the metrical thoroughness of the German school or the statistical enthusiasm of the American.

Jacquetta Hawkes does not perhaps evaluate as highly as she might this particular achievement, although she describes Wheeler's individual excavations fully and well. It deserves to be set in a wider context. For it would be quite possible to see Wheeler's keen sense of problem in fieldwork as anticipating in some respects the deliberately problem-orientated approaches of the "New Archaeology", which grew up in the last decade of Wheeler's life. It is not the inclination of either approach to set great store on facts just for their own sake. Both see the aim of fieldwork as the verification or testing of hypotheses through the gathering of fresh material, and recognize that it is ideas and problems which should determine the excavation strategy. This clear sense of priorities makes Archaeology from the Earth an inspiring introduction to fieldwork; still the best introduction, in my view, although many new techniques have been introduced over the past 30; years. And while the author rightly stresses Wheeler's ability to imagine and bring to life the people behind the archaeological record, his encouragement of the technical specialisms of archaeological science whether conservation or radiocarbon

dating — and his recognition of their place within a more disciplined approach are at least as important.

The author's emphasis is perhaps a more personal one, although she copes well and expertly (being an archaeologist herself) with Wheeler's excavations and research. She is concerned to see Wheeler as a person, dealing as much with the private life (and many loves) as with the public achievement. She begins: "Mortimer Wheeler will rise from these pages as a Hero



Mortimer Wheeler — creator of a discipline.

figure. Of that I feel sure even as I write the very first words of my book". Certainly the remarkably varied career - yet singleminded in its devotion to archaeology — is admirably told. Her earlier chapters and description of the war years inevitably owe a great deal to the autobiography. But surprisingly, perhaps, it is the portrait of Rik Wheeler in his old age, as he is remembered by many today (including myself) which seems the most compelling, indeed moving. Those same qualities of panache, of awareness of the effect of the moment, which may have raised evebrows (and indeed aroused some enmities) earlier, and which caught the attention of millions of viewers in the 1950s, made him the most splendidly vivacious good company in his seventies and eighties, and a friend and encouragement to many half a century younger than he. They come over well in this affectionate portrait.

Wheeler came to archaeology with a military briskness of mind which helped him to make the subject, in the scientific as well as the organizational meaning of the term, a discipline. Perhaps just as important, he brought with him a zest and a gusto which made it, in a real and un-trivial sense, entertaining.

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Grounds for doubting the pessimists

P. D. Henderson

The Ultimate Resource. By Julian L. Simon. Pp.412. US ISBN 0-691-09389-X; UK ISBN 0-85520-440-0. (Princeton University Press/Martin Robertson:1981.) \$14.50, £9.50.

WRITING in 1933, Keynes said of Malthus's Essay on the Principle of Population that "it attracted immediate attention, and the warfare of pamphlets instantly commenced... which for 135 years has never ceased". Hostilities have continued during the half-century since Keynes wrote, and Julian Simon's new 400-page volume is a reminder that not only pamphlets are involved. The issues that Malthus raised so provocatively in 1798 are still live, unsettled and highly contentious.

The main issues are two: first, the extent to which it is possible for human societies to achieve material progress; and second, the effects of population changes on their prospects of doing so. In relation to these, it is still possible to think of the main battleline as drawn between Malthusians and anti-Malthusians, though as in other spheres of conflict there are wide differences of view within each of the two factions.

Following in the steps of the master, modern Malthusians are pessimistic on both issues, or at least inclined to emphasize dangers and limitations rather than opportunities. As to material progress, the possibilities for growth in output are seen as restricted in any given society, and for the human race as a whole, because of constraints on the availability of land or natural resources. From this arises the threat of overpopulation. It is admitted that population growth will create a larger labour force - more hands, as well as more mouths - and thus tend to raise the level of potential output; but given the constraints imposed by nature, not much can be expected from this positive effect. Thus higher rates of population growth will often be a drag on material progress. Conversely, lower population growth may hold the key to sustainable prosperity.

By contrast, anti-Malthusians take a more sanguine view. They are sceptical about limits to growth, while at the same time many of them stress the positive effects of population increase on a society's capacity to produce goods and services. These are the two main themes of Simon's interesting and readable book. The author pours scorn on current pessimism and prophecies of doom — despite one or two favourable references to Malthus himself — and the book may be described as a scholarly polemic.

Most of the argument is directed to the two issues identified above. Part One ("Towards Our Beautiful Resource Future") considers the possible scarcity of natural resources, and deals successively with minerals, food supplies, land, energy,

environmental pollution and conservation. On all of these Simon points out, with some well chosen and carefully presented statistics, that the evidence of recent decades is more encouraging than is generally realized. Thus minerals, food products and energy supplies have over the past century become less costly in relation to wages; world food supplies have grown more rapidly than population; while the expectation of life, which he regards as the best single indicator of pollution, has risen all over the world. In Simon's judgement, all these favourable trends are likely to be maintained into the indefinite future: in the case of the cost of raw materials, he makes an engaging offer to back his assessment with money, by accepting bets from readers who take a different view. He thus rejects completely the notion that economic growth is constrained by the availability or cost of resources.

Part Two is mainly concerned with the effects of population growth on average income per head. In most of the economiccum-demographic models that have been developed over the past 20 to 30 years, these effects are unfavourable, largely because it is assumed that with more children in the average family the rate of saving and capital accumulation will be lower, and that a higher proportion of capital investment will go towards duplication of facilities (more schools, for example), rather than increasing capital per head. As against this, Simon makes three main points. First, larger families may increase the willingness of individuals to save and invest. Second, there are likely to be significant gains from economies of scale, since total output will be higher. Third and most important, output per head will benefit from "the contribution of additional people to our stock of useful knowledge" (p.196): a larger population means not only more mouths and hands, but also more brains. His broad conclusion, which he argues is consistent with the facts of economic history, is that moderate as distinct from zero rates of population growth are likely to have favourable effects on income per head, at any rate in the longer run, in rich and poor countries alike.

In Part Three ("Beyond the Data") the argument is taken further than the issue of material progress alone, and other values are brought in. Simon holds that enabling an extra person to live and enjoy life has value in itself, and that the well-being of a society has to be defined with reference not just to the average standard of living of its members, but to their total numbers also. He makes a sharp attack on the values and assumptions of anti-natalist bodies, such as Planned Parenthood, and argues that there is no justification for trying to reduce rates of population growth, as distinct

from enabling parents to obtain the size of families that they would wish.

Throughout the book Simon's own position, as summarized just above, is contrasted with the latter-day Malthusians whom he wishes to discredit. There are numerous quotations, some from learned articles and monographs, but most from popular books, newspaper articles and advertisements, designed to illustrate the whole range of contemporary pessimism. The style is kept deliberately informal and colloquial, while the author's convictions are often set out in a highly personal way. Now and then this mode of presentation becomes a little trying, but in general the argument is clearly and effectively conveyed, without either condescension or oversimplification. In view of the range and complexity of the topics that are covered, this is a considerable feat.

In judging the merits of Simon's argument, two distinct tests are relevant, corresponding to the two aims which the book tries simultaneously to achieve. The first aim is destructive: to undermine and cast doubt on current neo-Malthusian pessimism. The second is to offer an alternative view of the world, an acceptable analysis of events, relationships and trends. Not surprisingly perhaps, the author succeeds more completely with the former task than with the latter.

. The process of demolition is carried out with skill and resource. In part, the prophets of doom are discredited by the various excerpts from their own works, many of which make sorry reading. But the main weight of the argument rests on the evidence of the past and on general economic reasoning, both of which are often neglected or undervalued by the pessimists, and which the author presents extremely well. Unfortunately, the views that he attacks are widely held and influential. By challenging them so effectively Simon has performed a real service.

When it comes to his alternative view of the world, there is more room for doubt. At a number of points the argument seems to me to be too unqualified or incautious, while in some respects it is less complete than it should be. For example, his generally admirable discussion of natural resources is marred by a failure to consider the possibility that oil is a special case. Because of what are often presented as facts of geology, it may be that the period of cheap oil was a historical accident, a bonanza which cannot be repeated. If this is so, and given the unique convenience and properties of petroleum as a fuel, a secular rise in the real price of oil is not at all to be ruled out; and it is insufficient to dismiss the increases of the 1970s, as Simon does, as being due simply to the machinations of OPEC. Moreover, because of its role in the world economy, and the uneven distribution of productive capacity and reserves, the effects of oil price increases are potentially much more damaging than in the case of other raw materials. Simon does not go into this, and his two chapters on energy are superficial.

As to population, a clearer distinction could have been made between two aspects of the question: the absolute size of a population, and its rate of growth. More space should also have been given to two arguments against rapid population growth: one is the possible congestion arising from higher densities, while the other is that a rapidly growing labour force may lead to a more unequal distribution of income. Again, Simon states correctly that a judgement on the net advantages of population growth may depend on how one weighs more distant costs and benefits in relation to those which are nearer in time. But he says nothing about how this valuation of time should be made, nor about the suitability of using for this purpose the actual rates of interest that are established in financial markets.

More worrying is an occasional tendency to rather serious overstatement. Thus in the concluding chapter, we are told that "The standard of living has risen along with the size of the world's population since the beginning of recorded time" (p.345). This is false. One can find phases of history — as for example in England from the mid-thirteenth century till around the end of the seventeenth — when a rise in population is thought to have led directly to a fall in real wages, and vice versa: the simple Malthusian model seems to fit the facts. It is probably only in the modern era that this inverse connection has been broken, first in the industrial countries and more recently in other parts of the world: possibly it holds good even now in some of the poorest and least developed countries. Here and at some other points of the argument, Simon has yielded to temptation: he makes overconfident generalizations about relationships that are subject to considerable variety and change.

Despite these reservations, the book is much to be recommended. It is possibly the best available modern treatment of the issues that Malthus raised.

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The hunting of the rorqual

Arthur Bourne

The History of Modern Whaling. By J.N. Tønnessen and A.O. Johnsen. Pp.798. UK ISBN 0-905838-23-8; US ISBN 0-520-03973-4. (C. Hurst, London/University of California Press: 1982.) £22.50, \$45.

THE POPULAR image of whaling as the harpooneer, lance in hand, standing bravely on the prow of a row-boat ready to do battle with Leviathan, is enshrined in the writings of authors like Melville and Bullen. The picture is a romantic one but, as both those writers knew, whaling was far from romantic. It was — and its rump is — a tough business, firmly based on hard commercial interests. If there is little romance in whaling, as compensation there is a world of fascination and of no period is this more true than when the new whaling industry was emerging from the old.

The modern epoch really began in February 1864 when a small steam-powered vessel, the Speset Fides, sailed out of Tønsberg in south-eastern Norway for the whaling grounds off Finnmark. It is the story of the unfolding of that modern history which is the subject of Den Moderne Hvalfangsts Historie, of which the volume under review is a much-condensed English translation. In it the authors have cleverly drawn together the strands of the technical, sociological and financial developments of that period.

The birth of the modern whaling industry will always be associated with one man, the owner and skipper of the Spes et

Fides, Svend Foyn. Why he was to be the leading force and why Norway should have been the birthplace are interesting questions. As the authors show, the Americans dominated the old whaling scene and were experimenting with new methods at the same time as Foyn. Even his idea of combining an explosive harpoon fired from a cannon mounted on a steampowered boat was not original, and he was not the first man to realize that the future of whaling lay in the chasing, catching and processing of the great rorquals. The real answers to those questions lie not only in the technical inventiveness of the various contenders - of which there were many but also in the social and economic changes that were occurring at the time.

In the middle of the nineteenth century the Americans were looking increasingly inwards. The opening of the West with its vast prairies and potentially enormous mineral wealth seduced capital and labour away from the coast and the sea. In Norway the situation was very different. With little hinterland and an increasing population which the nation's industry and agriculture could not sustain, thousands of Norwegians, in common with many other Europeans, sought their futures in North America. By one of those quirks of fate, America's retreat from the sea opened up an opportunity for the Norwegians left at home. They were quick to fill the gap, and, combined with the expansion of sea-trade generally, this provided the stimulus for the growth in Norway's maritime industry.

At the same time the economics of whaling were propelling technical developments along parallel lines in several countries. The stocks of right whales -"right" because they were slow swimmers and floated when killed - had been so decimated by the middle of the century that they were unable to support a viable industry. The interest of the whalers turned to the rorquals which "teemed in the seas", but which were too swift for the row-boats and, moreover, sank when killed. One of the first to experiment with explosive harpoons was the American T.W. Roys. who invented a harpoon-throwing gun and set out to see "if a cargo of oil could be obtained from whales previously unavailable to mankind". There were other innovators and other developments, one of which was, the authors believe, of decisive importance to Foyn's own ideas. This was the grenade harpoon invented by George Welch in 1867, three years before Foyn's final patent was granted. In many countries similar inventiveness was displayed, but Foyn was the most persistent. He believed in the potential of the rorqual fishery even during his experiments with the Spes et Fides when failures lost him money, he kept going. He was in the end the right man at the right time.

The first of the rorqual stocks to follow the right whales to extinction was, not surprisingly, the Finnmark stock. From then on it was for the whaling companies a matter of geographical expansion and greater sophistication in techniques and ship design. The whalers extended their activities to wherever rorquals could be found and eventually to the waters surrounding the Antarctic continent. It was there that the greatest harvests were to be culled, and where the technical ingenuity of the whalers was again stretched.

The problems were to stay at sea long enough to justify the cost of fitting out a fleet, and to build more powerful and faster catchers. The floating whalefactory, heralding the pelagic whaling industry, extended the range of the catchers, and the introduction of offloading the oil into tankers enabled the fleets to remain at sea for months. From 1937 and the launching of the Japanesebuilt Seki Maru, the catchers became progressively more powerful. However, no matter how long the whalers were on the oceans, nor how sophisticated their equipment, if the whales were not there they could not be caught. Inevitably the Finnmark story was to be repeated again and again, and nation after nation, including Norway, had to abandon pelagic whaling - political ingenuity in conserving whale stocks fell far short of the technical expertise in destroying them.

The whaling industry is neglected by the student of the development of the industrial nations and yet it reflects the social and economic conditions of the times most vividly. It also provides clear insight into the way those conditions forced men to seek means of utilizing resources that had hitherto been out of reach. The story of the whaling industry shows too how difficult it is to maintain an industry based on natural resources, even renewable resources, if immediate commercial considerations over-ride the long-term benefits of rational exploitation and investment.

The authors of this book have achieved a remarkable piece of condensation and R.I.

Christophersen must be congratulated on a fine translation. The only sad thing about this volume is that it is unlikely that we shall ever see an English-language version of the original four-volume work — and that it is equally unlikely that mankind will learn from the mistakes it so amply documents.

Arthur Bourne was an observer at the International Whaling Commission from 1964 to 1968. His great-grandfather was a contemporary and competitor of Svend Foyn.

Cartography: from astrolabe to orbiter

Helen Wallis

The Mapmakers. By John Noble Wilford. Pp.414. UK ISBN 0-86245-041-1; US ISBN 0-394-46194-0. (Junction Books/Knopf: 1982.) £9.95, \$20.

A NOTABLE theme in the history of human endeavour is the record of man's survey and mapping of the Earth, the Moon and (by spacecraft) of the nearer planets. Even the earliest surviving records show a surprising degree of sophistication in the cartographic arts. Thus an example of early cadastral mapping has been identified in the depiction of a village found in rock carvings of the Valcamonica in northern Italy and dating from the second and first millennia BC. The Babylonian world map of about 500 BC is one of the earliest depictions of the cosmos. And two silk maps recently recovered from the Ming tombs show the advanced level reached by the Chinese in the second century BC

With the development of the geographical sciences in classical Greece, the coordinate system for the mapping of the world was established which is still the accepted method today. Even in the so-called "dark ages" of Europe, a continuing tradition can be traced from the surveys of the late Roman empire through mediaeval times.

The European Renaissance brought about a revolution in mapmaking. The newly invented techniques of engraving made possible the "exactly repeatable picture", and in the late fifteenth century led to the establishment of the mapmaker's craft and the map publishing industry. At the same time European ventures of exploration set in motion the mapping and surveying of the world. A comparable revolution has taken place in the past 50 years with the development of aerial surveys, automated cartography, and mapping from and in space.

The history of the complicated processes involved in these developments can be told in various ways. The role of the mapmakers themselves is often subordinated to the record of the artefacts, the atlases, maps and globes. John Noble Wilford in his new history of cartography sets out to correct this bias. In The Mapmakers he tells the story of "the multitude of diverse characters", ranging from scholars and scientists to clockmakers, schoolteachers and spies, aviators and technicians, who stand out in the history of cartography. Although he has not spent a lifetime working with maps as such, his professional post as science correspondent for The New York Times makes him well qualified to identify the pioneers and innovators in the history and explains his concern to bring out the drama of personal triumph. As professional mapmakers normally write their reports in "the bloodless language of the specialized



Surveyors at work in the eighteenth century — detail from a map of Warwickshire by Henry Beighton, 1728.

journals", he supplements written sources for modern surveying with interviews with the practitioners themselves, at NASA, the ÚS National Geodetic Survey and so on.

No single volume on the history of cartography can be comprehensive. It is important therefore to note that Wilford's book is really a history of the attempts to measure, survey and map the Earth, the Moon and the planets. The definition of cartography implied in this treatment is that of the United Nations, therefore, and not that of the International Cartographic Association (which excludes survey from its terms of reference). What is particularly valuable is the integration of the historical and the modern. Most histories of mapmaking end in 1900 or earlier, and leave to others the recording of recent developments. Thus the first two parts of the book deal with the broad trends and signal achievements of cartography and geodesy prior to the twentieth century. The final two parts include some earlier history but are focused on the twentieth century.

Another, less welcome, aspect of the treatment is an evident "Western" bias. Like many other histories, the book is "ethno-centred" (to quote the current term), dealing largely with the achievements of Mediterranean and European peoples. Arab geographers such as Idrisi, for example, are conspicuous by their absence, and the remarkable achievements of Chinese cartographers appear only in the pages dealing with the maps from the Ming tombs. In so focusing the story Wilford follows Lloyd, A. Brown, whose Story of Maps (Little, Brown; 1949) provided the classic account of mapmaking to 1800. The similarity in treatment and in the selection of illustrations for the early period explains why some parts of Wilford's book (for example, the sections on the Cassinis and John Harrison) will seem almost familiar to those well acquainted with Brown. Wilford's detailed account of the survey and mapping of India and North America extends the compass, however, in addition to the fact that the time span covered by Wilford is much greater.

The book is illustrated by reproductions of some of the most important maps and by photographs, but documentation of the illustrations is inadequate. The Babylonian world map (p.11), for example, is in the British Museum, not the New York Public Library (p.415), as the reader might suppose from the credits. Sources for information in the text are, for the most part, not cited, but bibliographical notes are provided for each chapter.

In general, however, Wilford's Mapmakers can be warmly recommended as an essential addition to library reference shelves, and as an excellent introduction to mapmaking past and present for geographers and map lovers alike.

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Ancient woods in modern times

John Andrews

Woodland Conservation and Management. By G.F. Peterken. Pp.328. ISBN 0-412-12820-9. (Chapman & Hall: 1981.) £25, \$49.95. Ancient Woodland: Its History, Vegetation and Uses in England. By Oliver Rackham. Pp.402. ISBN 0-7131-2723-6. (Edward Arnold/University Park Press: 1981.) £50, \$99.95.

DURING the past 30 years, more ancient mature woodland has been destroyed in Britain than in the preceding four centuries. The direct cause of this has been the parsimonious attitude of post-War government towards forestry and a corresponding benevolence to agriculture, with an increasingly phoney system of cost-benefit appraisal favouring overproduction from farming while squeezing forestry into short-rotation, softwood monocultures whose main product is pulp.

It is a tribute to the Forestry Commission's technical skill that it has succeeded in developing techniques of wood production under uniquely difficult conditions in the uplands, but no credit to foresters in general that they have fostered the destruction of Britain's remaining native woodlands, both by encouraging their replacement by conifers and by meekly acceding to their conversion to farmland. Perhaps conservationists too must share the blame, for failing to protest sooner and louder: not least the Nature Conservancy Council which should lead the movement, and yet still shies away from producing a policy on forestry after nearly a decade of indecision.

Fortunately, many NCC staff are a good deal harder-nosed than their politically-appointed Council. Woodland Conservation and Management is written by George Peterken, a member of NCC's scientific team, and ventures some provocative views. Thus,

private landowners, who enjoy a disproportionately large share of national wealth, should be prepared to bear a disproportionately large share of the sacrifices needed to provide national needs for amenity and nature conservation. Indeed, the continued political acceptability of private landowning may depend on recognising these and other public responsibilities

— a view gaining currency among conservationists patronizingly advised to leave protection of the countryside to the good will of the landowning community.

The book, however, is primarily a scientific work. It commences by examining the origins, management and ecological characteristics of British woodland, demonstrating the special value of ancient, primary woodland to a wide range of species which have persisted in such sites — and nowhere else — despite many centuries, perhaps in some cases several millennia, of human management.

The second part of the work considers the types of semi-natural woodland, their classification and management. Woodland classification in Britain is still bedevilled by the lack of a generally accepted system and here the effort is made to present one for use by any competent naturalist or forester. Such a classification has an important practical value, providing the basis for identification of woodland sites of similar type so that comparative conservation and management judgements can be made.

About a third of the book is devoted specifically to woodland nature conservation. There is much helpful advice on the recording and assessment of woodlands and on management methods, both where conservation takes priority and where the first objective is production. Though the emphasis is on native woodlands, the pros, cons and opportunities presented by the new conifer forests in the uplands are not neglected.

The book is well and economically written. Though some sections are technically complex, it contains much of general interest, not least a clear and concise statement of the objectives and priorities of nature conservation - matters' on which many foresters and some naturalists seem confused or misinformed. However, as an ornithologist, I must regret the author's heavy botanical bias: though birds have great powers of dispersal, many survive best in ancient woodland; and the absence of any reference to Britain's only endemic bird - the Scottish crossbill, which appears to be largely confined to Caledonian pine forest — cannot be lightly. forgiven.

In Ancient Woodland, Oliver Rackham, approaches the subject from a different standpoint. In the author's words, the main theme is

the self-renewal typical of English woodland history: the quiet, usually unremarked regrowth of the young shoots, automatically replacing the woods every time they were cut down, decade after decade and century after century.

But it is more than that. Dr Rackham's special skill lies in unravelling man's relationship with woodlands and, in scholarly yet entertaining style, he deduces from them aspects of rural social history since pre-Roman times.

Though the book contains examples from many parts of England and the Continent, it is based largely on the author's own studies in East Anglia. This region is a splendid study area, containing an unusual variety of woods which have been well documented — paradoxically the outcome of early clearance which meant that the remainder assumed special importance for local use and was systematically conserved until recent years.

Roughly the first quarter of the book covers woodland classification, soils, plant and tree communities; in some measure this overlaps with Peterken but the emphasis on man's role in shaping the woods gives a usefully different slant. Evidence from pollen analysis, soil structure, earthworks and archaeological remains, vegetation and tree rings, oral tradition, paintings and written records is brought together to present woodlands not only as places of beauty, rich in wildlife, but also as a part of our heritage - as much a reflection of past human skills, aspirations and shifts of power as any cathedral.

Moving on to the human uses of woods, the author leads us from Neolithic times through the extensive records of Domesday to

the mad world of the 1970s, in which the price of ordinary oak-trees is as low, in relation to the

value of money, as at any time since the fifteenth century; the price of oak *timber* is higher, again in relative terms, than at any time in history; and prices paid for underwood, provided the seller knows where to find the buyer, seem to be roughly equal to the previous all-time maximum in the eighteenth century.

Nearly half of the text deals with individual tree species, examining their distribution, prehistory and history, modern ecology and conservation. With many references to specific sites, one is presented with the opportunity to go and see for oneself — a temptation I will certainly find irresistible when the sun shines, just as dipping into the book will be a continuing pleasure on rainy days.

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, Bedfordshire. that heart disease hardly occurred in Roseto, Pennsylvania, while the inhabitants maintained a cohesive. conservative society, although they ate a typical Western diet. Wolf himself in Social Environment and Health (University of Washington Press, 1981) views disease as maladaptation to changing social conditions. Inglis concludes that it is therefore wrong to treat disease by orthodox methods. He proceeds to select the failures of modern health care and to ignore all the successes. He would have us believe that all heart surgery is a wasted resource, that no cancer patients benefit from chemotherapy, that mental hospitals should be abolished and that immunization plays little role in preventing infectious diseases. He states that "the net value of orthodox medical treatment is far less than it was twenty-five years ago, and is declining".

The chapters I find most objectionable. no doubt because they deal with my own subjects of study, are on infectious diseases and on cancer. Inglis dismisses research into the identification and control of infectious agents in disease (for example in Legionnaires' disease and in malaria) as "mechanistic heresy" obscuring psychosocial elements. He also shows a remarkable prejudice against virologists. imagining that they narrowly pursue infectious organisms without reference to epidemiology and pathogenesis. Elsewhere he supposes that virologists have sought to monopolize cancer research, preventing immunologists investigating "cancer immunotherapy", whereas it is the virus-induced cancers that may yield first to immunological means of prevention or therapy. The eradication of smallpox, the resounding medical triumph of the 1970s, is not cited at all in Inglis's book, yet its success depended on the very combination of preventive vaccination and thorough epidemiological investigation that orthodox medicine provided where 2,000 years of ayurvedic methods in India failed. The successful outcome of virological research leading to immunization against poliomyelitis earns a grudging mention, with the extraordinary suggestion that the rare but devastating neurological damage caused by polio virus infection is more related to psychosocial stress than to the virulence of the virus strain. Polio and infectious mononucleosis are two particularly intriguing diseases of civilization in that children of the higher social classes are the ones most at risk; the epidemiological factors underlying this phenomenon are not probed by Inglis, presumably because they do not fit his view of how virologists conduct research.

Inglis's criticisms of orthodox cancer treatment resemble those which have recently occupied the columns of the Washington Post and the diatribe by Dick Richards (The Topic of Cancer: When the Killing Has to Stop; Pergamon, 1982) against those who "cut, burn and poison"

The malaise of modern medicine?

Robin Weiss

The Diseases of Civilisation. By Brian Inglis. Pp.371. ISBN 0-340-21717-0. (Hodder & Stoughton: 1981.) £10.95.

BRIAN Inglis is a respected journalist and author who has written several informative books on the history of medical practice, orthodox, homeopathic and supernatural. His latest, The Diseases of Civilisation, is subtitled "An indictment of traditional methods of medical treatment and a plea for a completely new approach". Inglis surveys the major diseases affecting the Western world today - heart disease, cancer, mental illness, common infections, "auto-immune" and degenerative diseases - and concludes that modern medical science and practice has failed to live up to the high hopes of the immediate post-War period. Rather than question how realistic these expectations ever were, generated by journalists like himself as much as by the medical profession, Inglis concludes that the medical establishment is to blame for the intractability of ill-health in modern times. He depicts the medical profession as a self-perpetuating oligarchy composed of too many narrow specialities which dominates medical education and research, distorting both to serve its own ends and thereby obstructing public health. The food, tobacco and pharmaceutical industries are the accomplices in this medical crime, abetted by Governments that shy away from confrontation with an entrenched medico-industrial complex. Inglis argues that this unholy alliance has prevented the medical profession from embracing and acting upon discoveries of the important roles of diet, stress and other social factors in the causation of disease, so that resources which should be channelled into primary health care are deflected into the ever-increasing expense of high technology hospital treatment.

Much of this argument is regrettably only too true. The medical establishment is extremely conservative and profoundly suspicious of social or "unorthodox" approaches to disease. As Thomas McKeown has long argued, medical education is almost entirely geared to the treatment of acute illness as presented in teaching hospitals; the underlying causes of disease, other than specific pathogens, receive scant attention and the very concept of health is hardly raised during the training of doctors. Governments faced with butter mountains and tobacco revenues usually duck environmental and health issues when confronted by powerful lobbies - witness President Carter dismissing Mr Califano from the Department of Health, Education and Welfare, and Mrs Thatcher reshuffling Sir George Young out of the Department of Health and Social Security as soon as they tried to take significant action on curbing the promotion of cigarette consumption.

Brian Inglis has tackled a most important subject — the limitations and misconceptions in the modern Western approach to illness and health — yet I find his book disappointing and his message pernicious. Posing as a carefully researched documentary, it is more a polemical tract in the tradition of Ivan Illich's *Medical Nemesis* in which Inglis overstates his case to the point of being anti-science. His main thesis is that psychosocial stress is not taken into account in the investigation, management and prevention of diseases of civilization. He cites Stewart Wolf's important finding

cancer patients. Few would deny that patients sometimes receive excessive or inappropriate treatment; but having berated specialities, Inglis does not see that this is most likely to happen when cancer patients are treated by non-specialists. Inglis blames the medical establishment for the lack of progress in treating cancers of the lung and breast. Yet he makes no reference whatsoever to the successful treatment and cure of many paediatric cancers and leukaemia, Hodgkin's disease, choriocarcinoma or testicular cancer, which all result from the development of the aggressive forms of therapy which he so derides. True, these are rare types of cancer in terms of total incidence of malignant disease; but they are major causes of serious illness in children and young adults, with a high social and personal toll. There are common cancers too — of the skin, for example — for which diagnosis at an early stage undoubtedly reduces mortality very significantly. Inglis also entirely ignores palliative treatment, assuming that surgery, radiotherapy, chemotherapy or endocrine treatment invariably cause more suffering than leaving the cancer patient untreated by orthodox methods.

Inglis's account of cancer research funding policy is also seriously misinformed. He states that because the British cancer research charities "are largely controlled by specialists, naturally they favour projects which help them in their hospital work, providing them with better-equipped operating theatres, improved radiotherapy machines, more powerful drugs". This is nonsense, as no research project is more difficult to obtain funding for than one that smacks of a medical service role. Following the British Medical Journal's peculiar outburst two years ago, Inglis reiterates that less than 2 per cent of the British charities' expenditure is directed towards research into cancer epidemiology and prevention, an absurd underestimate even if one discounts the potential of basic research for future prevention. He thinks the public should be given more opportunity to influence the way in which cancer research funds are spent. This is a laudable sentiment, though difficult to implement;

Handlist for historians

The Science Museum, London, has recently published a vade-mecum for science historians, Reference Books for the Historian of Science: A Handlist. The bibliography has been compiled by S.A. Jayawardene with the intention of providing a volume to complement other bibliographic manuals, covering the primary and secondary sources of the history of science, by the inclusion of general reference books. The handlist includes some 1,000 titles, and is available from Library Publications, Science Museum Library, South Kensington, London SW7 5NH, price £2 50, £3 by post

moreover, "the public" will almost always choose to fund high-technology hardware, such as CT scanners, in preference to research into the psychosocial aspects of cancer that Inglis rightly wishes to promote.

In criticizing the medical management of the mental patient Inglis is on stronger ground, in that no single orthodoxy has emerged. From his account one does have the feeling that the theory and practice of treatment for mental illness has not advanced beyond the blood-letting era. Having heaped scorn upon the medical establishment's "obsession with diagnosis" for infectious diseases, Inglis here deplores the unreliable classification of mental disease, quoting large discrepancies between US and British psychiatrists. In fact, the WHO definition of schizophrenia can now be used to standardize diagnosis. As Julian Leff discusses in his recent monograph Psychiatry Around the Globe: A Transcultural View (for review see p.523), the incidence of schizophrenia is surprisingly uniform across different cultures, though its recognition as a disease state requiring treatment differs widely.

Inglis misses another opportunity for serious debate in his chapter on iatrogenic disorders. Quoting the Hippocratic dictum "At least, do no harm", he does not discuss whether the harm induced by treatment might be less than that of no treatment. Inglis justly reproaches the medical profession over drug abuse and carefree prescription, particularly in treating the symptoms of diseases of civilization without paying due attention to underlying causes. Yet in an earlier chapter he criticizes by-pass surgery for angina pectoris when chronic drug treatment might achieve the same result. One of the most notorious examples of iatrogenic disease (not quoted by Inglis) was the induction of leukaemia in patients treated with X-irradiation for ankylosing spondylitis. The radiotherapy undoubtedly caused enormous relief from the symptoms of a crippling, painful and eventually fatal hereditary disease; given the prospect of that relief, with the possibility of dying from iatrogenic leukaemia 15 years later, would Inglis have advised the spondylitis patient to spurn treatment?

Overall, Inglis has broached an important topic by questioning the basis of our current medical philosophy and its disregard for preventative health measures and social care. But I wish that he had not chosen to present orthodox medicine and research as the antithesis of genuine care and that he had researched his commentary more deeply. More importantly, I earnestly hope that any young man with testicular cancer or parent with a child suffering from leukaemia will not refuse orthodox treatment as a result of reading this book.

Robin Weiss is Director of the Institute for Cancer Research, London.

Politics of pesticides

M.S. Swaminathan

Circle of Poison: Pesticides and People in a Hungry World. By David Weir and Mark Schapiro. Pp.99. ISBN 0-935028-09-9. (Institute for Food and Development Policy/Third World Publications: 1981.) \$3.95, £2.50.

Circle of Poison provides an insight into the process by which "someone in the underdeveloped countries is poisoned by pesticides every minute". The circle begins with the activities of leading multi-national corporations which dominate the sevenbillion-dollar-a-year pesticide market. The links in the chain include loopholes in government regulations in the United States, the import policies of developing countries and the indifference of bilateral and multilateral donors, regional and international banks and UN agencies.

Weir and Schapiro argue their case cogently. For example they point out that at least 25 per cent of US pesticide exports are products that are banned, heavily restricted or have never been registered for use in the United States - "The Federal Insecticide, Fungicide and Rodenticide Act explicitly states that banned or unregistered pesticides are legal for export". Developing countries which receive hazardous pesticides recommend the application of such pesticides in a routine manner, partly due to ignorance and partly due to their being cheaper than less-toxic products. An instance has been cited where parathion is being used in Central America at a dosage 40 per cent greater than necessary. In contrast, through the adoption of better pest-management procedures, US farmers now use 35-50 per cent less insecticide than ten years ago, with no adverse effect on crop yield. An important consequence of the indiscriminate use of pesticides is the multiplication of pesticide-resistant insect species, leading to the use of still higher concentrations of the chemical, and so on.

At each stage in the process, the circle of poison has its victims - the staff in pesticide manufacturing plants, those who load and unload the chemicals, Third World peasants, workers and consumers, and finally everyone else in the world who eats food contaminated with pesticide residues. But undoubtedly the greatest impact is felt in the developing nations. According to some calculations, the rate of pesticide poisoning in such countries is more than 13 times that in the USA. The authors give examples from their investigations in Mexico, Central America, Pakistan, Indonesia and Papua New Guinea to illustrate how pesticides prohibited in the US reach the peasants of the Third World. Unfortunately, in these countries there are neither enough scientists to investigate the dangers arising from such pesticides nor is there a wellinformed and well-equipped government machinery to prevent foreign pesticide makers from selling products which are banned in their home countries.

However the United States and other pesticide exporters also suffer the consequences of the dumping of banned chemicals in developing countries. According to the US Food and Drug Administration, about 10 per cent of the food items imported into the United States contain higher levels of pesticides than are permitted. Therefore, even on the basis of enlightened self-interest, it would be advisable for developed countries not to permit the export of chemicals which cannot be sold at home.

The authors also question the pathway of productivity improvement involved in "the green revolution strategy". By encouraging monoculture, losing genetic diversity and not promoting integrated pest-management schedules, the use of large quantities of pesticides has become essential. The authors suspect that the emphasis on this kind of high-yield technology is largely because of the interests of the multi-nationals — several of them are involved not only in the production and sale of pesticides, but also in the production and distribution of seeds and chemical fertilizers.

The circle of poison has become possible only because of a circle of collusion between national and international companies, banks and government and UN agencies; all playing a role in the spread of poisonous chemicals. Fortunately, in recent years, some steps have been made towards breaking the unholy triple alliance of pesticide manufacturers, importers and finance institutions. The authors propose several methods by which the fight against the proliferation of hazardous pesticides can be brought to a successful end. Action, they say, has to be taken both at national and international, and individual and institutional levels. Examples are cited of the kinds of effective checks being developed in the Philippines and Malaysia. The resolution of the United Nations General Assembly passed in December 1979, urging member states to exchange information on hazardous chemicals (and pharmaceuticals) that have been banned in their countries, and to discourage export of those products to other countries, is another move in the right direction. This has been followed by similar steps by OECD and other organizations. The Environmental Protection Agency in the USA is also becoming more vigilant.

David Weir and Mark Schapiro have rendered signal service by drawing attention to the deficiencies in existing regulations and practices in the trade of hazardous pesticides in both the exporting and importing countries. The book, therefore, deserves to be widely read by all connected with agricultural research and development as well as those involved in the development of public policies relating

to human health and environmental protection. Nonetheless, their account does have some flaws.

In the section entitled "More Food and Yet More Hunger", the authors try to place the blame for rural poverty mainly on developments in the improvement of agricultural production. This is not wholly correct since it is now widely accepted that the new technology involving the cultivation of management-responsive varieties is neutral to scale with regard to the size of the farm in which the technology is adopted. Hence, a small farm is not a handicap from the point of view of adoption of new technology. A small farmer, however, has many problems arising from the cost, risk and return structure of farming. The potential offered by a small farm for intensive agriculture can, therefore; be realized only by removing the constraints faced by small farmers. The action required is in the realm of public policy and not technology. Technology can only help to maximize

output from the resource endowments of each country; poverty can be minimized only through appropriate political decisions in the fields of land and livestock reform, asset transfer and social security. Unfortunately, terms such as "miracle seeds" have created the impression that new technologies can provide miraculous solutions to problems of poverty, social inequality and injustice. It would have been prudent to have avoided the temptation of making a few casual and over-simplified statements on rich-poor equations, since such problems deserve in-depth analysis. This would have lent greater authority and credibility to the principal message of the book — that the temptation to make profit out of poverty and ignorance can be countered only through widespread awareness of the techniques used for this purpose.

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Whence the power of the medicine-man?

Robert Ubell

The Clay Pedestal: A Re-examination of the Doctor-Patient Relationship. By Thomas Preston. Pp.226. ISBN 0-914842-68-4. (Madrona: 1981.) \$12.95.

THOMAS Preston, the author of this book on modern medical practice in the United States, is a cardiologist with his heart in the right place. Acutely aware of the way doctors play musical chairs with their patients, he rises to their defence, sympathizing with the patients, castigating the physicians.

Preston's analysis closely follows much of what many of us already know from sad experience. Divorced from their patients, concentrating on their business, toying with medical technology, doctors often alienate the sick and frightened with impoverishing bills, high-handed manners and bureaucratic mazes. He lays the blame for these failures on elite training at medical schools, authoritative and paternalistic attitudes, and the advantages of power, prestige and money. Arrogant doctors, he claims, frequently act decisively when hesitation and doubt are best; often they perform no better than quacks and faith-healers.

From greed, doctors tend to over-treat and over-test:

When a surgeon is faced with a choice between doing nothing for which he collects a thirty-dollar consulting fee, and recommending surgery for which he would get three thousand dollars, is he not going to be influenced in the direction of surgery? Only a physician would dare suggest impartiality under the circumstances.

Preston cites studies showing that Americans who come to their doctors for help are twice as likely to undergo surgery as their British cousins. Since the overall mortality rate in the two countries is roughly the same, either Americans are more prone to illness - which seems unlikely — or a vast number of pointless operations are being performed. And because of over-medication and useless therapies, many patients become ill some even die - because they visited their doctor. Had some stayed away, allowing healing to take its natural course, they would have remained alive and well, unharmed by iatrogenic disease.

Preston's most cutting attack is against doctors who practice medicine as if it were a guessing game, relying solely on their subjective clinical judgement rather than proven scientific methods. The random, controlled trial is the only sound basis on which doctors can claim effective therapies; yet physicians continue to walk down unscientific paths. Preston shows that medicine without science is doomed to repeat its recent and dangerous errors radical mastectomy, chelation treatment for arteriosclerosis, pure oxygen for premature babies, electroshock therapy for mental illness: Ironically, it was under the banner of improved scientific standards that physicians campaigned for and ultimately won control over the practice of medicine in the United States.

Presson draws parallels between American doctors today and ancient healers, taking us back to tribal communities where the shaman and the primitive medicine-man dispensed their arts. He tries to show us how in fundamental ways medical practice has remained unchanged for millennia, that the basic needs of patients and the old ways of practising medicine emerge as a natural consequence of social interaction. He argues that unless patients rise up and fight for their rights, doctors, who always have had the upper hand, will continue to exercise unreasonable control.

His argument from history is, however, sketchy and flawed. Anthropologists tell us that healers in tribal societies did quite different things in different cultures for their sick and dying. Levi-Strauss reports that the "shaman" in one society may act the self-conscious charlatan, reaping the rewards of power and prestige; in another, the healer may be someone who merely happens to know tribal lore. Moving on, Preston also forgets to inform us that mediaeval barbers and surgeons practised their trades in the same guild. Nor does he tell us that, until modern times, surgeons and chefs were in the same fraternity in the British navy. What bound the cooks, barbers and surgeons was not their natural social standing, but their knives and scissors — or perhaps the fact that food. hair and disease have always been and will always be with us.

By making us believe that the doctorpatient relationship is a natural feature of the human landscape, Preston stumbles and falls into the same myth-making apparatus that holds us all in its thrall. While he makes some recommendations for reform, he fails to see how he has adopted the same myths that have helped American doctors secure their power and privilege.

Preston would have been a better guide had he not turned the pages of his history book so far back. In the nineteenth century, American physicians practised alongside homeopaths, midwives and assorted fakers and purveyors of patent medicine. It was not until the early days of this century that doctors, under the political leadership of the American Medical Association, codified their position and under the slogans of standardization and faith in science, systematically excluded all but those who emerged from approved medical schools and who gained access to practice by passing state licensing exams (also controlled by the AMA and its local societies). By the 1920s, the AMA had established a complete monopoly over the practice of medicine in the United States.

In The Clay Pedestal, Thomas Preston has tackled a subject which demands close and critical scrutiny. But an analysis of medicine that merely reflects what patients already feel about their doctors, and neglects to tell them how doctors gained their power, says a lot about what we already know and nothing about what we do not.

Robert Ubell is the American Publisher of Nature.

Around the world of mental illness

G. Morris Carstairs

Psychiatry Around The Globe: A Transcultural View. By Julian Leff. Pp.204. ISBN 0-8247-1532-2. (Dekker: 1981.) SwFr. 58.

This conspectus of mental illness "around the globe" has many of the attributes of those luxury cruises in which travellers combine the pleasures of exotic sight-seeing with those of being instructed by experts about what they have seen. Dr Leff is an admirable tour guide. He handles statistics with respect but is always ready to point out their limitations as well as their contributions to knowledge. As befits an epidemiologist, he first scrutinizes the methodology of any survey and only then discusses its findings.

His global tour de l'horizon is clearly presented, under four major headings: (i) Do psychiatric conditions look the same in different countries? (ii) Do psychiatric conditions have 'the same frequency in different countries? (iii) Are psychiatric conditions treated differently in different countries? (iv) Do psychiatric conditions have a different course in different countries?

These four parts are followed by a final section discussing the mental health of immigrants in general, and of West Indian and Asian immigrants in Britain in particular. He gives us interesting summaries of research findings in each of these areas of inquiry. From start to finish one basic question keeps recurring, namely: if multiple culture-specific factors enter into the perception, treatment and outcome of psychiatric illnesses, then how meaningful is it to make cross-cultural comparisons of these illnesses?

There is now ample evidence that both organic and functional psychoses can be identified in every society; but even in respect of these major illnesses we have to proceed cautiously in interpreting symptoms in the same way as we would do in the West. As Leff puts it: "It is clear that delusions and hallucinations, the main symptoms of psychotic illness, can only be judged as present in relation to the patient's cultural milieu". This still leaves us uncertain as to just how such symptoms are to be evaluated.

A large part of this book is thus devoted to demonstrating ways in which the procedures of psychiatric diagnosis are being made more valid and reliable than they have been in the past. An interesting example is the US/UK Project which had its origin in an observation of the biostatistician, Morton Kramer. He pointed out that, if their respective data were to be believed, then the first admission rates for manic depressive psychoses in the age group 55 to 64 were twenty times higher in Britain than in the USA. Could this really be true?

In order to put the question to the test it was necessary to construct an objective, systematic interview schedule and to train a team of six psychiatrists (four British and two American) in its use. The instrument chosen was Professor John Wing's Present State Examination (PSE). The team used it to ascertain the "project diagnosis" for consecutive series of patients admitted to hospitals serving London and New York. When they did so, the previously wide discrepancies in diagnoses became very much reduced. Analysis of the results showed that hospital doctors in New York were accustomed to use a much wider, concept of schizophrenia than that used in London, as a result of which they included under the label of schizophrenia many patients who would be diagnosed as manicdepressive in London.

This was the first international use of the PSE. Its next major deployment was in the WHO-sponsored International Pilot Study of Schizophrenia (IPSS) in which teams in nine countries (the USA, Britain, Colombia, Czechoslovakia, Denmark, India, Nigeria, Russia and Taiwan) collaborated in collecting cohorts of patients who met certain defined criteria, assessing them in terms of the PSE and then following them up for a period of five years. Here the PSE could be used in its original form only in Britain and the USA; in each of the seven other countries it had to be translated into the local language.

This international study (which still continues) has produced some interesting findings; among them, the demonstration that patients with specified "core symptoms" of schizophrenia could be found in every country; and also that the final diagnostic judgements (whose criteria were not laid down) were fairly uniform in seven countries but widely divergent in the other two, namely the USA and Russia, In. the former, a cultural emphasis on Freudian concepts led to a wide concept of schizophrenia so that many cases were included under this heading which would not be so regarded in the other countries. In Russia, diagnostic practice was — and still dominated by Professor Snezhnevsky's theory that schizophrenia presents in three forms, much attention being paid to the postulated course of the disease. Many patients were diagnosed as schizophrenic because although they did not yet show major symptoms it was predicted that they would eventually do so.

Dr Leff is candid about the large obstacles which hinder cross-cultural agreement about certain of the symptoms of mental illness, particularly in the neuroses. He devotes a chapter to discussing "The language of emotion", pointing out that there are some languages in which there are no single terms for "depression" or "anxiety". In translating

an instrument such as the PSE into Yoruba, for example, recourse has to be made to metaphors in common use such as "the heart is weak", for depression, and "the heart is not at rest", for anxiety. One cannot be sure that these phrases correspond at all precisely with the English words.

Not only are some languages richer than others in the language of emotion: even within cultures members of underprivileged groups may have less well-developed abilities to recognize or to express nuances of feeling (as Basil Bernstein has demonstrated in British society).

Dr Leff is dedicated to the use of standardized instruments, accompanied by glossaries and sets of instructions as the best way to enable different observers to recognize and record symptoms in the same way. Usually he remains keenly aware of the difficulties which still remain, but very occasionally he lapses as when, after completing a review of a number of surveys of psychiatric illness in Asian countries he draws attention to the wide differences in the prevalence rates for neurosis, and comments: "We can infer that the very highest rates found in the Asian surveys are likely to represent the most realistic estimates of the true prevalence of the neuroses".

Normally, he would be the first to question the concept of the "true prevalence" of neuroses. He knows that minor neurotic symptoms are very common in normal subjects. Determining at what levels of frequency and severity these symptoms should constitute an illness is a judgemental decision. Until some less subjective indicator can be found, the prevalence of neuroses can only be measured in terms of stated, hopefully relevant, operational criteria. As yet there is no such thing as the "true prevalence" of neuroses — and perhaps never will be.

The chapters on the mental health of immigrants to the USA and to Britain are interesting not only for their findings (that, in general, immigrants show a high rate of incidence of schizophrenia but that Asian immigrants attend their general practitioners for minor complaints less often than do their British neighbours), but also because they show that useful crosscultural research can now be carried out within the British Isles and USA. One factor, however, appears to have been overlooked. This is Srole et al.'s report, Mental Health in the Metropolis: The Midtown Manhattan Study (McGraw-Hill, 1962), which found very high rates of psychiatric disorders among immigrants but only among poor immigrants. The much smaller number of rich immigrants did not experience an excess of these disorders.

The final chapter tells us, in grim detail, about the many handicaps which an Asian immigrant physician has to contend with in trying to advance his career in a Western country: these are economic, linguistic,

social, educational as well as professional. Having taken a special interest in the welfare of overseas postgraduate students at the Institute of Psychiatry, London, Dr Leff knows what he is talking about; but here he seems to present a totally gloomy picture. Every experience of the immigrant doctor is a negative one, culminating for some in a neurotic or psychotic breakdown, with all their disastrous implications. Is there no gleam of light at all in the experience of our immigrant trainees? Surely we can all remember some exceptions, men and women who have earned the friendship and respect of their British or North American colleagues and have embarked upon an academic or clinical career. Admittedly, they are in the minority. This chapter, although tangential to the rest of the book, will serve a useful purpose by reminding us about the difficult lot of our overseas trainees.

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Art of insects

J.L. Cloudsley-Thompson

Insects Etc.: An Anthology of Arthropods Featuring A Bounty of Beetles. Pp.108. ISBN 0-933920-25-3. (Hudson Hills: 1981.) \$50.

What is there in common between Edgar Allan Poe, Jonathan Swift and Raoul Hausmann? The answer, immediately revealed by perusal of the literary anthology with which P.A. Gette enlivens this unusual book, is that they were not good observers of insects. Like Salvador Dali's pre-1950 ants, Hausmann's houseflies had only four legs, Swift's were endowed with stings and Poe confused bugs with beetles. Far more observant were Lewis Carroll and Vladimir Nabokov and, of course, Réaumur, Thoreau and Jean-Henri Fabre. Buffon was evidently a careless writer: "The insect is a small animal

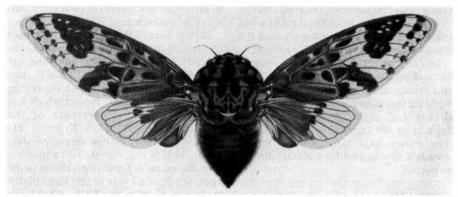
without blood. The latter [sic] can be divided into large and small kinds".

Literary activities, like art, are not necessarily marred by inaccuracy such as this. Less excusable, however, are the errors of nomenclature and spelling apparent in the names of the various arthropods portrayed. For instance, Argiope bruennichi (mis-spelled) is an impressive, striped, orb-web spinner, but it is not the zebra spider. This name has always been reserved for Salticus scenicus the little jumping spider, common on walls and fences throughout Europe, Asia and America. Again, the scorpion is not Buthus occitanus as stated but, unmistakably, Scorpio maurus, the classical "scorpio" of antiquity.

The 34 dazzling, large-format colour plates are taken from photo-realistic paintings by Bernard Durin, without doubt an unusually gifted illustrator in the great tradition of Audubon, Edward Lear and Louis Agassiz Fluertes. Of 33 species illustrated (there is a close-up of the head of Zonocerus variegatus in addition to a painting of the whole grasshopper), 16 are beetles, 5 grasshoppers, 6 Hemiptera, 3 Hymenoptera, a single butterfly (the peacock), a spider and a scorpion. The species depicted are, as might be expected, large, brightly coloured and sometimes bizarre. The paintings illustrating them are breathtaking. Durin is not only a meticulous observer but he really succeeds in creating the illusion of irridescence in his beetles and in making insects' wings look transparent. A commentary on each of the arthropods illustrated, with information about various aspects of its behaviour, life cycle, food, enemies and folklore, is provided by Gerhard Scherer.

Zoologists are often so familiar with the subjects of their research that they overlook obvious questions posed by them. What are the functions of the extraordinary colours of some insects, why do others have such long legs or antennae, what are the advantages and drawbacks of their shapes and sizes? It might be worthwhile taking a second, thoughtful look at the subjects of Durin's paintings.

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Pycna antinorii, the African cicada, painted by Bernard Durin. Reproduction in black and white and reduction in size do scant justice to the picture.

REVIEW ARTICLE

Mantle metasomatism—continuing chemical change within the Earth

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Mantle fragments carried up from as deep as 200 km by high-velocity eruptions show metasomatic introduction of volatile bearing minerals, before melting. The broad spatial and temporal distribution of alkaline magmatism shows that transfer of lithophile elements to the outer Earth by rising volatiles, is a long-term and continuing process in planetary evolution.

METASOMATISM is a chemical change, whereby a pre-existing mineral or rock is converted to another composition. It usually refers to a solid-state transformation, with material transfer through a vapour or fluid, without melting. Paradoxically it has always found easiest acceptance when applied to lowtemperature and -pressure phenomena, for example, the detailed replacement of calcium carbonate fossils by iron sulphide, or the substitution of plant tissue by silica in fossil wood. Mining geologists have long accepted metasomatism as an important process, giving rise to a whole class of metasomatic or 'replacement' ore bodies, spectacular examples of which are wholesale replacement of limestone beds by metal sulphides¹. Obviously, some evidence of the original material must remain as proof of metasomatism, and with higher temperatures and pressures this becomes more vulnerable. Controversy is most likely when deep metasomatism is proposed as the origin for a relatively common rock such as granite²: the end product is chemically unexceptional, and the issue complicated by rock recrystallization (metamorphism) at high temperatures and pressures. Even so it should be noted that most metamorphic changes are not isochemical because the mineral reactions involve at least volatile losses or gains3.

On a limited scale, metasomatic replacement in crustal rocks is well documented. , new minerals formed including feldspar, tourmaline, kaolin, micas, sulphides, serpentine, carbonates, calc silicates, alkali amphiboles and pyroxenes. All cases involve volatile or mobile elements from the range K, Na, C, H, F, Cl, S and B, and the metasomatic process operates by fluid infiltration or diffusion. The efficacy of processes of this kind is demonstrated by the oxygen isotope equilibration between large rock masses and circulating groundwaters⁷. Nonetheless, as a major rock-forming process in the crust, metasomatism may still be controversial, so that mantle metasomatism (where the evidence is fragmentary) might seem esoteric. The concept arose from the need to integrate geological, petrological and geophysical observations on cratonic alkaline magmatism^{8,9}. This concept was supported by the fact that crustal rocks intruded by alkaline magmas provide perhaps the best examples of alkali metasomatism, now known as 'fenitization' from the type area at Fen (Southern Norway)¹⁰. Subsequently, mantle fragments brought up by alkaline volcanism were found to be metasomatized, such that if melted they would yield the compositions observed as lavas11. Support came from chemical studies on alkaline lavas from oceanic islands, indicating variations in the source mantle that would be explicable by alkali enrichment before melting¹².

Composition of the mantle

The mantle constitutes the major part of the Earth (84% of the volume and 68% of the mass) and is the chemical and energy reservoir driving crustal and lithogenic processes. As most of the information about the mantle, and all below 250 km,

is derived from geophysical observations, the picture that emerges is inevitably smoothed by the limited resolving power of the indirect observations. Furthermore, remote physical measurements are unlikely to discern anything less than gross variations in chemistry (and even then the domains of chemical variations would have to be very large). Consequently there is some danger of treating the Earth's mantle as if it were chemically homogeneous or (perhaps even more dangerous) rendered homogeneous soon after accretion of the proto-Earth. For convenience it is customary to regard the bulk composition of the mantle as being essentially peridotitic (dominantly magnesium-rich silicates, olivines and pyroxenes) similar to the silicate fractions of stony meteorites¹³, particularly the abundant chondrites. Meteorite compositions are widely used as reference standards for minor (trace) elements and isotopes, but the once popular chondrite Earth model has (with some exceptions¹⁴) largely fallen into disfavour¹⁵. An internationally agreed standard for bulk mantle chemistry would have value in general geochemical comparisons, but any chemical reference standards are liable to abuse in the sense that they can all too readily assume the status of a real piece of mantle from which specific surface rocks were derived. Garnet peridotite, as brought to the Earth's surface through diamond pipes, is the preferred choice for a generalized bulk composition 13,16 and although Ringwood prefers, as a geochemical device, to use a hypothetical material, pyrolite, (a chemical mix of peridotite and basalt) he notes that garnet peridotite is "rather similar in composition"15. Beneath this apparent unanimity there is diversity, ranging from the "bulk composition of the mantle is very uncertain" to there is "major element homogeneity throughout the mantle source regions" of basaltic magmas¹⁵ It is, however, largely from the difficulty in explaining the variations in chemistry between magmas that concepts of mantle heterogeneity have grown¹⁷. Some types of heterogeneity go beyond those expected from irreversible changes caused by previous expulsion of melts to the surface, and seem to require additions or mixing of sources earlier in the Earth's history.

Arguments for metasomatic changes in mantle composition fall into two categories—those deduced from chemical characteristics of lavas, and those where there is observed replacement of an earlier mineralogy. Both need to be seen in a geological context. Direct observation has claim to precedence but it is convenient to deal with lava chemistry first, because the case has been developed in the less complex framework of oceanic volcanism, and because the two lines of investigation become most compelling in unison.

Chemical variations in the oceanic mantle

All the evidence for mantle metasomatism is provided by igneous activity, especially mafic (basic) and ultramafic magmatism for which there is abundant evidence of origin at least in the upper mantle, and maybe deeper. As liquid samples from

the mantle; the magmas themselves may be expected to carry some imprint of the source chemistry. Deciphering the imprint depends on simplifying assumptions, some of which may be easily acceptable, but all are debatable. Basic lavas erupted within the ocean basins are generally believed to be uncontaminated by continental crust and surface materials. Because of their voluminous and widespread eruption, mid-ocean ridge basalts (MORB) are taken to be the products of high degrees of melting of the source mantle, and to have many chemical characteristics that are unmodified by subsequent crystal fractionation. Isotope ratios of heavy elements such as Sr, for example, are held to be immune¹⁸⁻²⁰, but all these views have been challenged²¹. Most geochemists working on MORB, however, are satisfied with the assumptions as a working hypothesis, which allow the use of element concentrations, ratios and isotopes to characterize the chemistry of the source mantle. Trace elements that because of their ionic size and charge cannot be accommodated in the crystal lattices of the main peridotite (mantle) minerals are said to be 'incompatible' and hence strongly partitioned into any melt that may form. Using crystal-melt partition coefficients it is possible to calculate expected fractionation patterns of various elements, for different amounts of melting of plausible combinations of mantle minerals. The calculated patterns may then be compared with those observed in MORB. The first attempt gave the best fit for the MORB incompatible element patterns at 15-30% melting of a model mantle peridotite, whereas alkali basalts from oceanic islands seemed to be restricted to as little as 3-7% melting if they were to give the required patterns²². Also the higher concentrations of a range of large ion elements in alkali basalts, compared with MORB, required that the source mantle was richer in these elements than the MORB source²². Gast postulated that the mantle supplying alkali basalts was more primitive, and hence the MORB source had lost some of its large ion elements in an earlier melting episode. He described the MORB source as "depleted" with respect to the supposedly primitive alkali basalt source. Behind this notion of depletion was the knowledge that there is commonly insufficient of the large ion radioactive isotope ⁸⁷Rb in MORB to support the observed amounts of the daughter isotope 87Sr, which is most readily explained by some Rb loss in the earlier history of the mantle source. Subsequently, it became clear that by the same token ocean island alkali basalts had too much Rb for their content of ⁸⁷Sr, and this was consistent with their source having been 'enriched' in large ions some time before eruption²³. In current usage, 'enriched' often means just 'more abundant',

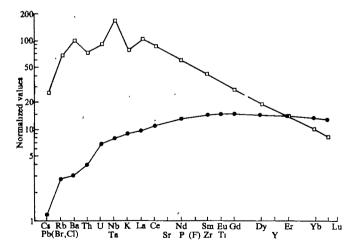


Fig. 1 Comparison of normalized abundance patterns of incompatible elements from alkali basalt (□) (oceanic islands) and midocean ridge tholeiite basalt (●). Adapted from ref. 25. Refractory lithophile elements such as rare earths, Ti, Zr, U and Th normalized against chondritic meteorites. For other normalizing values see ref. 25. Highly alkaline lavas such as nephelinite will in general plot at higher levels than alkali basalt.

and may even start to assume the connotation of 'metasomatized'. Before describing something as 'enriched' or 'depleted' its previous condition must be known. This leads back to the most difficult assumptions, concerning the origin and evolution of the planet. Typical are discussions about rare earth elements in layas, in which the abundances are compared by normalizing against a chondrite meteorite standard. For example, the lower concentrations of the light rare earths (La, Ce, Pr, Nd) in MORB, causing a downward dip in their distribution pattern when normalized against chondrite abundances, may be said to be due to an earlier depletion in the mantle source. Taken alone this conclusion can only be based on an expectation that an undepleted mantle source would provide liquids with light rare earth distributions similar to, or higher than in chondrites: either way, assumptions have been made about the source, and a real (but unseen) segment of mantle has been invested with some of the attributes of an arbitrary geochemical standard. This can lead to bewildering mixtures of hypotheses and observations, but without making any assumptions about the previous history of the mantle, it is still possible to deduce from the chemistry of lavas that their sources were different at the time of melting. More and more geochemists have favoured heterogeneity in the mantle to account for the differences between fresh lavas of similar major element chemistry, when these have been erupted contemporaneously in the same region¹⁷. In favourable circumstances it may be possible to conclude in favour of heterogeneity even on the basis of major element ratios in lavas, for example, Mg/Fe (ref. 24), but most cases are based on the range of incompatible elements depicted in Fig. 1.

If chemical heterogeneity in the oceanic mantle is accepted, the debate can turn about the nature and extent of the variations, and their cause and timing. Often the most that can be deduced from lava chemistry is that the sources were richer or poorer in particular elements by comparison among the samples, or against some chosen standard. By adding isotope systematics, it can be argued that the observed lava isotope patterns cannot be explained by undisturbed isotopic evolution in the source throughout the entire history of the Earth (Fig. 2). This proposition is made more plausible by the difficulty in conceiving of a segment of mantle that could have remained totally isolated for 4,600 Myr, but note that the isotope systematics are based on meteorite abundances and must rest on assumptions about isotopic distributions in the primordial Earth. If it is accepted that the systematics define the expected behaviour in a closed system within the Earth, the departures from the geochron indicate that the isotopic development of a particular piece of mantle has been disturbed. Depletion then usually means Rb deficiency, and is almost always ascribed to earlier episodes of melt extraction from primordial, primitive, or fertile mantle. Similar deductions have been made for Nd/Sm (ref. 27), Th/Pb, U/Pb and Pb isotope distributions: diagrams similar in principle to Fig. 2 may also be constructed, but Nd/Sm data are less abundant, and the complexities of the Pb systems require more extensive coverage for satisfactory presentation²² Taken in concert the isotope patterns in a lava can make an impressive case for a complex history of its mantle source²⁵

Enrichment (such as Rb excess) implies source mixing, the simplest interpretation being that two sources have been mixed, but the nature of the mixing, the number of mixes, and the real number of sources, are matters of conjecture. It might be that melts from deeper sources have become lodged in and contaminated higher mantle levels, which are then subsequently remelted 12.28-31 or a variety of melts have mingled in their passage to the surface, maybe as a complex continuum. Alternatively, fluids from another mantle source have contaminated melts at higher levels 2 or metasomatized the mantle through which they pass 3. The lava erupted at the surface may be the product of any combination of such processes, or others as yet unknown. But all that the 'disturbed' isotope patterns in lavas reveal is that the mantle sources have not had a simple history. Careful integration of different chemical evidence, such as

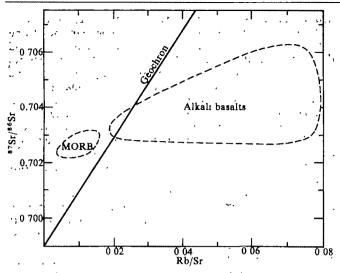


Fig. 2 Rb-Sr isochron diagram showing fields of alkali basalts and MORB. The 4,600 Myr isochron (geochron) indicates the distribution that would be expected from isolated (closed) mantle domains that had the same primordial ⁸⁷Sr/⁸⁶Sr as basaltic achondrite meteorites (BABI), but different Rb/Sr when the Earth was formed. Compositions left of the line have Rb insufficient for, and those to the right Rb in excess of, the amount necessary to support their observed ⁸⁷Sr, if the geochron prescription is correct. The simplest explanation is that the Rb-Sr systems in the mantle sources have not remained undisturbed, suggesting loss of Rb from the MORB sources and gain by the island volcano sources. From refs. 12, 26.

combined isotopes, trace elements and element ratios, can be developed into a forceful argument for source enrichment before melting³⁰. With improved geochemical understanding, and more information, it may be possible to discriminate in favour of enrichment by volatile activity, and metasomatism may then be the most plausible process; but the ultimate proof of metasomatism can be imprinted only in the mantle solids.

Volcanological evidence

Mafic and ultramafic lavas form the bulk of the material for geochemical conclusions in favour of mantle metasomatism, and it is therefore ironic that often the geology or volcanology of the specimens is accorded little or no weight in the argument. Those igneous rocks with the highest contents of incompatible elements have long been referred to by petrologists as 'alkaline', so that abundance of K and Rb, two of the prime incompatible elements in modern discussions, is already implicit. Alkaline magmatism, oceanic and continental, is characteristically expressed in central volcanoes and igneous complexes, giving rise to fragmental, explosive and sometimes high velocity eruptions³⁴. It is these last that carry up ultramafic fragments (nodules), providing solid samples of the deepest available mantle. Furthermore, fast eruption allows little opportunity for modification of the melt as it rises to the surface, and provides some of the best samples of mantle melting. Other incompatible elements are thus manifest in alkaline igneous activity, namely the gases which are the cause and driving force of high speed eruption. Furthermore, the gases in this magmatism are distinctive, especially in their contents of CO₂, Cl and F (refs 34-36). Therefore richness in alkalis (and other incompatible elements) and gases is no coincidence8, and was a prime reason for proposing mantle metasomatism as an essential part of alkaline igneous activity9. This does not say that the volcanological evidence is anything other than circumstantial (as indeed is the geochemistry of lava samples), simply that in considering the case for mantle metasomatism all the evidence should be weighed:

Against this volcanological background, the petrographical evidence of metasomatism in mantle nodules brought to the surface by igneous eruptions should be viewed.

Petrographical evidence

To know that metasomatism has happened, the evidence must be seen in the rock, and solid samples of mantle are available only as volcanic xenoliths, and peridotite masses in fold mountain belts. The latter may have been emplaced as relatively cool tectonic slices, or hot intrusions, forced through the Earth's crust. Some contain veins of less refractory minerals, and veined peridotite has been held up as one exemplar of 'enriched' mantle that might yield alkali basalts by preferential melting of the vein material 30,37,38. The veins have been interpreted as formed by melt injection, or segregation. Evidence of metasomatism while the peridotite was still in the mantle is not yet available, and may not be easy to distinguish from low-pressure changes after emplacement in the crust. Hence the only petrographical evidence of metasomatism is in mantle nodules of volcanic derivation. Even here careful observation is necessary to eliminate the possibility that the changes did not result from reactions between a peridotite fragment and the high temperature, volatile-rich melt which brought it to the surface.

Peridotite nodules consist dominantly of olivine and orthopyroxene with, or without, small amounts of clinopyroxene, and/or spinel, and garnet. It is these last three minerals especially that contain the Ca, Al and Na, and are consequently important contributors to basaltic liquids, the most common surface products of mantle melting. Spinel peridotites are typically carried up in alkali basalt (basanite) eruptions, while garnet-bearing varieties are much more restricted, and largely confined to strongly silica-unsaturated volcanics, such as melilitites, and especially kimberlites. All these nodule carriers are notably rich in incompatible elements, but even tholeiitic basalts have K and H₂O contents that are hard to account for by realistic degrees of melting of peridotite unless it contains small amounts of an additional phase, such as amphibole³⁹ or phlogopite mica⁴⁰. Experimental studies⁴⁰⁻⁴³ indicated that these minerals had appropriate stabilities for existence in the upper mantle, but early reports of amphiboleand mica in peridotite nodules were circumspect, either because the minerals seemed secondary (and formed outside mantle conditions)44 or because the peridotite nodules might be magmatic crystal accumulations (and not accidental inclusions picked up by the magma)⁴⁵. But evidence was mounting in favour of most peridotite nodules being mantle samples and textural studies soon left little doubt that in some peridotites, phlogopite⁴⁸ and amphibole⁴⁹ had grown in equilibrium. These hydrous minerals were seen as either an integral part of the mineralogy of the mantle sampled by the eruption 48,49, or formed as a result of igneous veining around a magma body in the mantle⁵⁰. In one case it was suggested that direct melting of amphibole peridotite could have produced the nephelinite melt that brought the fragments to the surface⁴⁹.

Mica and amphibole are the two most characteristic metasomatic minerals in peridotite nodules, the process being recognized by intricate partial replacement of the earlier minerals. New minerals invade the old in delicate lobes and embayments, until the stage may be reached where only scattered (but undisturbed) residuals remain: the rock is pervaded by infiltration ('seepage') of new material along grain boundaries, and even along cleavage planes in the existing minerals¹¹. Clinopyroxene also forms metasomatically, its composition being distinctly different from any that may be present in the original peridotite^{51,52}. A suite of samples can show all stages from incipient metasomatism of peridotite through to complete transformation into mica-amphibole-clinopyroxenite. This latter rock has been found containing residuals of peridotite still intact¹¹: The minerals most susceptible to replacement are garnet, spinel and orthopyroxene, followed by olivine. Other new minerals introduced are carbonates, phosphate (apatite), and titanium and iron oxides and sulphides 11,53,54. By its very nature, metasomatism must yield varied end products, but the process may add K, Ti, Al, Fe, Mn, Ca, Na, H, C, S, P, Rb, Sr, Y, Zr, Nb, Ba and La (ref. 11), in different amounts. This is an impressive

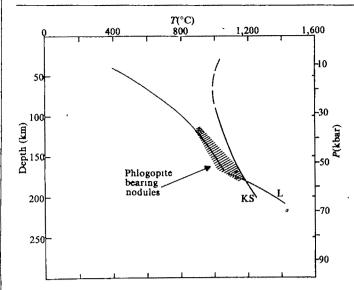


Fig. 3 Distribution of phlogopite-bearing mantle nodules from Lesotho kimberlites in relation to the geothermal gradient (L), and kimberlite beginning-of-melting (KS). Phlogopite has formed well outside the conditions where any melt can exist, and cannot be attributed to the action of magma. On the contrary, the melting curve may be the upper boundary for phlogopite survival in retrieved mantle samples. The distribution also suggests a maximum depth of ~200 km for metasomatized samples. Adapted from ref. 58.

array of incompatible elements, and the concentrations may also be remarkable: one opaque phase has been reported with 9.1% BaO and 7.4% ZrO₂. In addition to the pervasive alteration within the body of the rock, metasomatized peridotites are commonly cut by veins 11.53.54 containing assemblages of metasomatic minerals. These veins are truncated against lava at nodule margins, indicating that the nodules are fragments of larger masses disintegrated by eruption. In some cases there are small amounts of glass in the veins, with every sign that the liquid was in equilibrium with the vein minerals. This liquid, indicating the melting threshold, is in stark contrast with the host lava which corrodes and devolatilizes the hydrous minerals to clinopyroxene+olivine (±spinel)+glass 11.55. Furthermore, there are chemical gradients perpendicular to the veins within the nodules but not against the nodule margins 56. Thus, a range of observations rule out metasomatism of the nodules by the carrier melt at low pressure.

Not uncommonly the mica and amphibole in peridotite nodules is deformed^{54,57} in keeping with a time lag between its formation and nodule eruption. Furthermore, comparing mica bearing nodules with the mantle solidus on a PT diagram⁵⁸ (Fig. 3) shows that they equilibrated in conditions where no liquid could exist. Isotopic analyses of Sr in the metasomatic minerals and host nodules prove them to be out of equilibrium with the enclosing kimberlite, and indicate a significantly earlier metasomatic age⁵⁹. In other examples, although the metasomatized nodules and the recently erupted host lavas are in an isotopic harmony, the isotopic evidence requires the melts to be fusion products of mantle that had been metasomatized in an earlier event⁶⁰. Other studies reveal complex histories of changing chemical heterogeneity in the continental mantle, some of which were metasomatic, dating back to 3,000 Myr (refs 61, 62).

Conditions of metasomatism

Considerations of mechanisms (infiltration and diffusion) and the characteristics of the metasomatizing agents, are still in their infancy. Studies of the gaseous components of metasomatic amphiboles and micas indicate that the aqueous fluids coexisting with those amphiboles and micas brought up by alkali basalts were diluted by CO₂, O, F and Cl, while in samples from kimberlites all the H₂O may have been fractionated into

hydrous minerals⁵⁶. Experimental studies have already shown that fluids in equilibrium with synthetic phlogopite and olivine must contain considerable dissolved K, Al, and Si (ref. 3), and the agent of mantle metasomatism (whatever its precise nature) was clearly bringing in these and other large ion lithophile elements from greater depth. The ultimate, deep mantle source of the fluids is inaccessible, but the emission of gases from volcanoes is evidence of continual Earth degassing, and the key role of CO₂ in alkaline magmatism is of long standing³. Repetition of alkaline igneous activity in continental cratons predicates a persistent, or renewable, source of volatiles in the mantle^{34,36}. The same volatiles must be essential ingredients of the metasomatic process.

Figure 4 shows some experimentally determined mineral stabilities, and indicates that the most abundant metasomatic minerals in ultramafic nodules could have been formed by fluids moving through the mantle along typical geothermal gradients. Figure 3 shows this to be borne out by the metasomatized nodules in kimberlite pipes, and also indicates the expected upper boundary of the process—the onset of melting where the local geothermal gradients intersect the vapour-saturated solidus in the mantle. On low geothermal gradients, and with slow release of fluids from the deep mantle, metasomatism will continue and presumably affect a wider region, over a long period of time. But given a sufficient supply of fluid the channelways will become saturated with metasomatic minerals, and their temperatures will be raised, so that ultimately melting and the generation of alkaline magmas may be expected⁸. Such a regime explains the broad range of nodule types showing all degrees of metasomatism, and with some showing signs of melt formation. Upward leakage of volatiles can thus provide the means of chemical enrichment, and the trigger for igneous activity^{8,58}. A mechanism for volatile release and channeling through the lithosphere is shown in Fig. 5.

Volume relations

In fine detail the metasomatism in ultramafic nodules is nondisruptive, with undisturbed residual patches of the original host. At this scale part of the replacement may be equal-volume,

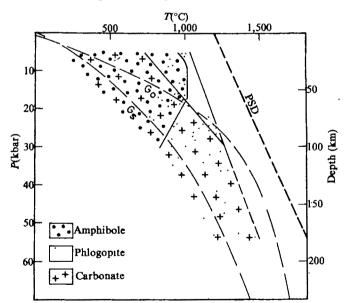


Fig. 4 Experimentally determined stabilities for possible metasomatic minerals, the boundaries (solid lines) indicating upper limits. Overlap of the stability fields cannot be used to infer necessary coexistence of minerals, which depends on factors such as bulk mantle chemistry, presence or absence of vapour (fluid), and fluid composition. Extensive mantle stability regions are indicated for continents (geotherm $G_{\mathfrak{g}}$) where alkali rich and carbonate magmatism are strongly developed. Mineral stability ranges, are much more restricted along typical oceanic gradients (G_0). PSD (heavy broken line) is the vapour absent peridotite solidus. Adapted from ref. 63. Experimental sources, refs 40, 43, 64, 65.

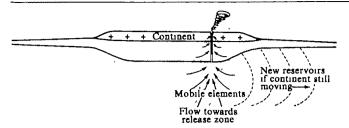


Fig. 5 Opening of a zone of weakness in the lithosphere allows volatile release to the surface. The escape channel effectively acts as a volatile and heat focus, leading to metasomatism and melting along the pathways. Adapted from ref. 34.

but it is almost impossible to rule out the possibility of rock distension along grain boundaries. Complete equal-volume metasomatism would require totally free exchange between the rock and the transporting medium, so that part of the original composition can be removed, as suggested in nodules from the Bultfontein kimberlite⁵⁴. In mantle conditions the bulk of any fluid entering a region where metasomatic minerals were stable would be consumed in carbonation and hydration reactions. Expansion is then unavoidable, making fluid escape zones selfextending by what amounts to metasomatic wedging. Consistent with this is the common observation of veining in metasomatized nodules. Once a crack system is open in the host rock. chemical exchange between fluid and solid must become easier, evidenced by the new minerals being relatively homogeneous. Complex zoning and other heterogeneities are common, however, in metasomatic minerals, indicating a spectrum of replacement conditions. Metasomatic veining itself requires some opening up of the host rock, even though most of the minerals along the veins may have formed by replacement. A parallel exists in alkaline metasomatism (fenitization) around alkaline complexes in the crust, where the outstanding physical characteristic is extensive shattering and veining of the host rock, often with little sign of deformation, or even block rotation. Mantle metasomatism requires that a pre-existing volume of dense peridotite has been changed to one that is larger and less dense, which explains the remarkable correlation of alkaline magmatism with uplift of the cratons (coupled with geophysical evidence of a lower density mantle)^{9,11}. Uplift by this process has the advantage of long survival, in contrast to uplifts of purely thermal origin. In fact the metasomatic uplift can be removed only by surface erosion or by subsequent thermal decomposition in the mantle: the latter may explain part of the subsidence observed in continental rifts when the axial igneous activity becomes established36.

Is metasomatism widespread or local?

First descriptions of metasomatized peridotites indicated two different modes for the process. On one hand the metasomatized nodules formed only a fraction of the total mantle sample in a kimberlite vent, and were attributed to local metasomatism by magma before eruption⁵³. At the other extreme, throughout two whole volcanic provinces large proportions of the ultramafic nodules were metasomites, sometimes to the exclusion of unaltered peridotites¹¹. Substantial parts of the sampled mantle in south-west Uganda and west Eifel (Germany) consist of alkali clinopyroxenite, and in the Uganda samples garnet and orthopyroxene are absent, and olivine not abundant. The lavas in these (and like) provinces have always been burdened by a plethora of names, giving little or no indication of their mineralogical affinities and obscuring the fact that in essence they are fine-grained clinopyroxenites. Their chemistry is appropriate to their being partial melts from alkali clinopyroxenite mantle: K-rich lavas in Uganda where the nodules are rich in mica, and sodi-potassic lavas in the Eifel where amphibole is more abundant in the nodules^{11,66}. A parallel case can be made for kimberlite, which in its earliest description was called mica peridotite. Nodule suites in which large proportions of the samples contain amphibole and mica are not uncommon, and there seems to

be a spectrum of conditions from extensively metasomatized mantle to a patchy or network distribution. Where metasomatism is extensive (and intensive) the lavas look like daughter products, and there is growing support for the view that metasomatism is the precursor of alkaline magmatism ^{34,60,67}.

Even a volcanic province such as south-west Uganda, however, is only a tiny mantle sample, and the need now is to see metasomatism in the context of the mantle as a whole. Clearly material is being introduced into the upper mantle from below, but is it only a local effect⁶⁸⁻⁷¹ and therefore of relatively small significance in mantle processes, or is it more widespread^{9,11,61}? Work on nodules from different kimberlites and of different ages across the southern African craton has demonstrated the use of coordinating different lines of enquiry⁶¹. Mineral textures show metasomatic replacement in nodules from many kimberlites, and a detailed Sr isotopic study of nodules from one kimberlite suggests they have a distinctly greater age than that of the host kimberlite (90 Myr), the metasomatic event being tentatively related to the Karroo magmatism (190 Myr) that affected wide areas of Africa and the rest of Gondwanaland^{59,72}. Metasomatism in nodules from the Precambrian Premier kimberlite indicates an earlier manifestation of the process in the same region⁷³. New Nd isotope studies are indicating two different episodes of metasomatism in nodules from the same kimberlite (Bultfontein) (A. J. Erlank, personal communication). More and more kimberlite pipes are being discovered in southern Africa⁶⁸, and although the volume of kimberlite in each case is tiny, the southern African craton has clearly been peppered with kimberlite eruptions, providing widespread sampling of the underlying mantle. Proportions of metasomatized nodules vary^{53,54} so the metasomatism is patchy in mantle sampled by kimberlite; but the time relations, and the spatial distribution, require that the process, although variable in intensity, is general below the southern African craton. Similar patterns emerge elsewhere, and the world-wide occurrence of kimberlite and alkaline magmatism bringing up mantle xenoliths containing amphibole, mica, and apatite suggests that metasomatism of the mantle, especially the subcontinental mantle, is widespread. The multiplicity of alkaline magmatism across the continents throughout their history indicates that metasomatism is not just an anomalous feature in an otherwise barren mantle. The age ranges, from Precambrian through to present, indicate a continual process, and the persistent association of metasomatized nodules with alkaline magmatism must surely mean that the metasomatizing fluids and the magmatism repeatedly exploit, as channels, long-lived weaknesses in the lithospheric plates⁷⁴.

Wider considerations

Geochemistry, and observational and experimental petrology show that the mantle is heterogeneous. Much of the Earth's thermal activity is expressed in submarine eruption at midocean ridges, and this has lent credence to the view that the continental upper mantle is largely depleted in its low melting constituents, and is barren, infertile, or effectively dead. Active volcanoes in continental interiors indicate a counter view, but their relative paucity perhaps causes them to be set aside as anomalies⁷⁵, or the progeny of mythical plumes. It is easy to overlook the fact that the cratons are the oldest parts of the Earth's surface, and since they became stable they have been repeatedly perforated, across their breadth, by alkaline igneous activity. Even tholeiitic flood lavas erupted on a regional scale may show significantly higher levels of incompatible elements than could be expected from a highly refractory residual mantle⁶¹, and some have chemical features close to that of the postulated bulk Earth 75 . The magmatism is a constant reminder that development of the continental mantle is far from complete. Repeated and widespread metasomatism of the mantle lithosphere would account for this activity, and would also be expected to contribute to continental growth by additions to the base of the crust^o. Continental permanence and constancy of crustal thickness could be maintained in the face of the fact that erosion now exposes Archaean deep crust at the surface. If the continental mantle is not exhausted, it raises the possibility that the special characteristics of the ancient cratons are inherited from original accretionary inhomogeneities in the formation of the Earth³⁴. Undoubtedly, the continental lithosphere still has challenges that can engage the scientific collaboration envisaged in the International Lithosphere Programme.

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Perhaps a start could be made by dismissing the view that the old continental heartlands had completed their evolution before ours began.

I thank A. J. Erlank, G. N. Hanson, F. E. Lloyd, M. A. Menzies and S.-S. Sun for support in the preparation stages, and for their stimulating work in this field; also A. J. Erlank and F. E. Lloyd read the first draft and gave helpful comments.

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Resolving crystallographically distinct tetrahedral sites in silicalite and ZSM-5 by solid-state NMR

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'Magic-angle-spinning' NMR has revealed fresh insights into the atomic structure of the polymorph of silica, silicalite, which has exceptional absorptive properties. There is evidence that highly crystalline variants of silicalite contain 24 distinct tetrahedral locations, populated principally by Si⁴⁺ ions, and that, contrary to what was originally thought, aluminium is also present, in at least two kinds of environments, in the tetrahedral framework. The silicalite structure is shown to be essentially indistinguishable from that of the siliceous zeolite catalyst ZSM-5.

THE new, thermally stable, crystalline phase of silica called silicalite described by Flanigen et al.1, possesses remarkable sorptive properties that stem partly from its exceptionally large internal volume (33% porosity) and partly from its three-

dimensional system of intersecting channels¹. It contains fivemembered rings in basic repeats (Fig. 1a) which are so arranged as to form a system of channels made up of 10-membered ring openings (Fig. 1b). Silicalite is hydrophobic, and it removes from water, by preferential adsorption, alkanols, certain alkanes and some other organic entities. It has been argued that

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"... unlike aluminium-containing zeolites, silicalite has no cation-exchange properties . . . silicalite has no aluminium and no cations in its structure". The original patent² asserts that such aluminium as may be adventitiously present is in the form of Al₂O₃. Although the details of its crystal structure remain to be fully clarified, it is established^{1,3-5} that, topologically, silicalite is closely similar if not identical to the shape-selective catalyst-ZSM-5, which because it selectively converts methanol to petrol, is a key component in the production of fuel from coal $(C+H_2O(steam) \rightarrow CO+H_2; CO+2H_2 \rightarrow CH_3OH)$. There have been claims4 that silicalite is an end member of the ZSM-5 substitutional series, which embraces Si/Al ratios from 10 to 4,000. But because it is extremely difficult, if not impossible⁵, to ascertain by X-ray crystallography whether aluminium in very low concentration is present substitutionally in the cornersharing tetrahedral (SiO₄) array that constitutes ZSM-5 and silicalite, there is still considerable debate as to the distinction between these two materials. We now show, by magic-angle spinning⁶ NMR, that: (1) several crystallographically distinct sites for Si atoms in the lattice may be directly detected by ²⁹Si NMR; (2) the aluminium in silicalite is readily detectable by ²⁷Al NMR; (3) the Si/Al ratio may be straightforwardly estimated; (4) all the aluminium is tetrahedrally coordinated to oxygens; (5) there are at least two distinct types of tetrahedral site occupied by the aluminium. We further show that there are 24 distinct crystallographic tetrahedral sites occupied by either silicon or aluminium in silicalite/ZSM-5, and that its

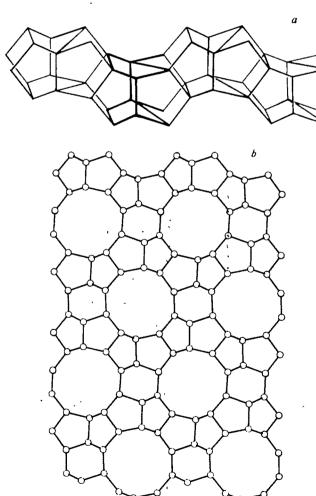


Fig. 1 The structure of silicalite. a, Secondary building units (indicated by bold lines) each composed of 12 Si atoms are linked into chains, one of which is shown in the c direction. b, The chains are interlinked to form a three-dimensional framework in which there are 10-membered ring openings (\sim 5.5 Å diameter) running along the [010] direction. In this portion of the ac structural projection, \bigcirc denotes a tetrahedral site.

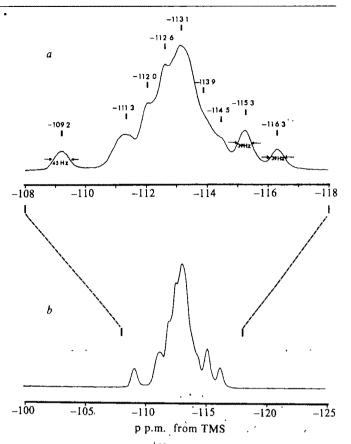


Fig. 2 a, b, High-resolution ²⁹Si MASNMR spectrum of silicalite at 79.80 MHz. 6,550 free induction decays were accumulated; repetition time 5 s. Chemical shifts are given in p.p.m. from TMS.

space group has lower symmetry than the currently accepted Pnma³. We conclude that, structurally, silicalite and ZSM-5 are essentially indistinguishable.

Structure determination

High-resolution solid-state 29 Si NMR with magic-angle spinning (MASNMR) is capable of distinguishing between the five possible Si(nAl) structural building blocks in zeolitic aluminosilicates, where n=0 to 4 is the number of tetrahedral Al atoms joined, by oxygen bridges, to the central Si atom⁷⁻¹⁰. In the spectra of crystalline synthetic faujasites (zeolites X and Y) for example there are up to five well-resolved signals falling within distinct chemical shift ranges; thus the value of n can be assigned to each signal, aiding structural elucidation¹¹⁻¹³.

By using ²⁹Si MASNMR at very high magnetic field¹⁴ on a sample of silicalite of very high purity and crystallinity prepared exactly as given in example 4 of ref. 2 but afterwards calcined in air at 500 °C for 16 h, we have obtained spectra of such high resolution that we can resolve separate ²⁹Si signals attributable to several crystallographically non-equivalent tetrahedral Si atoms, all joined, by oxygen bridges (denoted by straight lines in Fig. 1a, b), to four other Si atoms. These spectra, together with the corresponding ²⁷Al MASNMR spectra, provide new insights into the structure of silicalite.

Results

Figure 2 shows the 29 Si spectrum recorded at 79.80 MHz without using any resolution-enhancement techniques. The sharpness of the spectrum is exceptional, with FWHM of the resolved peaks of \sim 40 Hz (\sim 0.5 p.p.m.) which is superior to that so far observed in any other zeolite, even at this very high magnetic field. The complete spread of all the components of the spectrum (chemical shifts are given from external Me₄Si) is \sim 6 p.p.m. The chemical shift range of all the peaks (-112 ± 3 p.p.m.) is characteristic of Si(0A1) (that is Si(4Si)) groupings

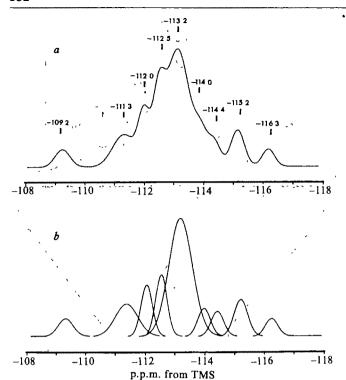


Fig. 3 The ²⁹Si MASNMR spectrum given in Fig. 2 can be computer-simulated using nine gaussian-shaped peaks, shown individually in b; their sum, simulating the spectrum, is given in a. The areas of the peaks in b are, from left to right, in the ratio 0.98: 2.70:2.19:2.63:10.35:1.30:1.16:1.87:0.82.

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in highly siliceous materials 7-10. The observed multiplicity arises from the crystallographically non-equivalent tetrahedral environments of Si(4 Si) silicons. The spectrum may be simulated by a minimum of nine gaussian signals, the intensities (peak areas) of which are approximately in the ratio 1:3:2:3:10:1:1:2:1 (see Fig. 3). Using the intensities of the well-resolved lowest and highest field signals as a base unit of one (that is we proceed on the plausible assumption that these well-resolved peaks each refer to one distinct tetrahedral site), the total intensity of the peaks in the Si(4 Si) multiplet is found to be approximately 24. This result suggests a space group which requires 24-non equivalent Si sites in the repeat unit of the structure. It signifies that Pn2₁a or P2₁/n (see below) are more appropriate than Pnma³ which has only 12 distinct tetrahedral sites. The relative intensities of the signals do not suffer perceptible change over a 10-fold increase in the delay time of the experiment, thereby indicating that they are quantitatively reliable. At this stage, it is premature to attempt to assign individual 29Si peaks to specific sites, particularly because

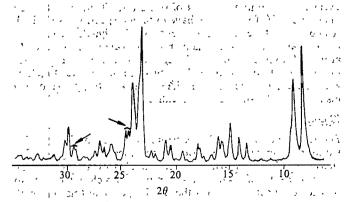


Fig. 4 Powder diffraction pattern of silicalite obtained on a Philips vertical goniometer using Cu K α radiation selected by a curved graphite monochromator in the diffracted beam. Peak doublets are arrowed.

interatomic distances in silicalite are not sufficiently well established (see footnote 11 of ref. 5).

Figure 4 shows a powder X-ray diffraction pattern of silicalite (the same sample as used in the MASNMR measurements shown in Fig. 2). Note the presence of peak doublets (marked with arrows) for 2θ values of 24.4° and 29.2° . Pseudosymmetry is common in zeolite structures and monoclinic as well as orthorhombic symmetry has been observed in ZSM-5-type materials. Wu et-al. simulated peak splitting by the introduction of monoclinic lattice parameters a = 20.17 Å, b = 19.93 Å, c = 13.42 Å and $\alpha = 90.64^{\circ}$, instead of the orthorhombic values of 20.07 Å, 19.92 Å, 13.42 Å and 90° , respectively. This small change in the unit cell causes the symmetry

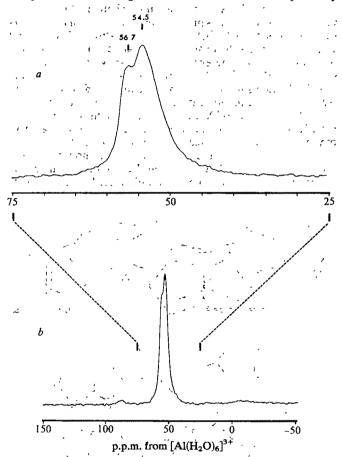


Fig. 5. High-resolution ²⁷Al MASNMR spectrum of silicalite at 104.22 MHz. 176,214 free induction decays were accumulated; repetition time 0.1 s. Chemical shifts are given in p.p.m. from [Al(H₂O)₆]³⁺. Note the absence of octahedrally coordinated aluminium in the sample.

elements such as the mirror planes parallel to (010) bisecting the zigzag-shaped channels to disappear; there are thus 24 (rather than 12) crystallographically non-equivalent Si atoms. The probable monoclinic space group for ZSM-5-type materials is, therefore, thought 15 to be P2₁/n. Change of symmetry occurs quite readily for ZSM-5 materials: indeed, a commercially available sample of silicalite exhibited an X-ray pattern identical to that shown in Fig. 4.

Discussion

The 27 Al MASNMR spectra show a rather broad absorption centred at ~ 55.6 p.p.m. with respect to external $[Al(H_2O)_6]^{3+}$, clearly characteristic of tetrahedral coordination 17 , both in this and in all other silicalite samples, which we have examined. In the sample the 29 Si spectrum of which is given in Fig. 2, the peak shows fine structure (Fig. 5) due to at least two components (at 54.5 and 56.7 p.p.m.) indicating the presence of crystallographically non-equivalent sites for tetrahedral aluminium. This agrees with the 29 Si spectrum in Fig. 2 and indicates that

the aluminium is tetrahedrally incorporated into the framework. Because of the 100% isotopic abundance of ²⁷Al and its very short spin-lattice relaxation time, even traces of aluminium are detectable by this sensitive technique. The aluminium in silicalite comes from impurities in the materials used in synthesis, and their level varies from sample to sample. The amount of Al present is small, but in all the samples of silicalite we have examined Al is tetrahedral and not present as occluded Al₂O₃ impurity² as the various forms of Al₂O₃ which we have investigated for comparison show an intense NMR peak at 9 ± 1 p.p.m. (reflecting octahedral coordination of Al) and in some also a small peak at 72±3 p.p.m. due to tetrahedrally coordinated17 Al, in agreement with structural data18 on yalumina. A quantitative determination by ²⁷Al MASNMR of Al concentration in the sample of silicalite was carried out by adding a known amount of a reference compound containing octahedrally coordinated Al and comparing the intensities of the two signals. This gives a Si/Al ratio ≥ 1,000. The aluminium content of the commercial silicalite sample was found to be higher (Si/Al \geq 200) and a small broad peak at -104 p.p.m. corresponding to Si(1Al) (that is Si(0Al) (0Si)₃ group) is clearly observed in the ²⁹Si MASNMR spectrum.

We have found general correspondence between the 29Si MASNMR spectra of silicalite on the one hand, and those for

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a variety of ZSM-5 zeolite preparations on the other. The latter show in the ²⁹Si MASNMR spectra an intense peak at about -112 p.p.m. with some indications of fine structure and a much smaller peak at approximately -105 p.p.m. corresponding to Si(1Al); and in the ²⁷Al spectrum a single peak at ~55 p.p.m., identical to that found at essentially this position in silicalite. The ²⁹Si spectra of ZSM-5 zeolites can, to a first approximation, be simulated by applying a large line-broadening function to the silicalite spectrum of Fig. 2, thus confirming the close? structural relationship between the two materials.

This work demonstrates the power of MASNMR as a structural probe for a large class of shape-selective aluminosilicate sorbents and catalysts.

We acknowledge support from the Universities of Cambridge and Guelph and from BP Research Centre, Sunbury. C.A.F. acknowledges the financial support of the Natural Sciences and Engineering Research Council (NSERC) of Canada in the form of an operating grant, G.C.G. the award of a NSERC Graduate Scholarship. We thank Dr D. Stewart, Dr B. Nay and Dr M. Barlow for making available the special preparation of highly crystalline silicalite. The NMR spectra were obtained at the South-West Ontario High Field NMR Facility (Dr R. E. Lenkinski, manager) funded by a Major Installation grant from NSERC.

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Monitoring of structural changes accompanying ultrastabilization of faujasitic zeolite catalysts

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By recording high-resolution solid-state NMR spectra of zeolitic samples spun (at ~3 kHz) at the magic angle $(\theta = 54^{\circ}44')$ so that $3\cos^{2}\theta - 1 = 0$ to the magnetic field, the chemical identity of the first and second coordination shells surrounding Si may be determined, and a distinction made between octahedrally and tetrahedrally bonded Al. The technique, therefore, enables the atomic course of dealumination, known to be responsible for the stabilization of catalytic zeolites, to be directly determined.

SYNTHETIC analogues of the rare mineral faujasite (Fig. 1) can be prepared in mild laboratory conditions such that materials with a wide range of compositions (zeolites X and Y with Si/Al ratios from 1.18 to 2.75) may be produced^{1,2}. The topology and overall crystallography of the aluminosilicate framework remain constant, but the population of Si4+ and Al³⁺ ions in the tetrahedral sites depends on the Si/Al ratio. These zeolitic solids, which are capable of cation exchange, possess large internal areas ($\sim 800 \text{ m}^2 \text{ g}^{-1}$) and volumes ($\sim 50\%$ porosity) and attractive molecular sieving properties. The diameter of the connected intracrystalline cavities varies between ~7 and ~13 Å. It has long been recognized that faujasitic zeolites are good catalysts for the cracking of petroleum; but in their as-prepared condition they proved

inadequate for hydrocracking, mainly because of their excessive acidity. A major turning point in the use of synthetic faujasites in catalytic cracking and hydrocracking came with the demonstration^{3,4} that on heat-treating NH₄-exchanged zeolite Y ultrastable catalysts were produced. These materials can withstand temperatures of ~1,000 °C, retain their internal surface areas and exhibit desirable hydrocracking activity. Over 200,000 tons of ultrastable zeolites are now produced annually: faujasitic catalysts are the cornerstone of the petroleum industry.

It is known from chemical analysis, and indirectly from the reduction of the unit cell dimension (monitored by X rays) that the Si/Al ratio increases markedly, a fact which both improves the stability and quells the acidity. But what are the atomic rearrangements associated with the process of ultrastabilization? There has been much speculation: it has been surmised

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that the dealumination gives rise to detrital aluminous entities. In what form is this produced; and which are the groupings in the parent zeolite that are demolished?

Magic-angle spinning NMR

²⁹Si and ²⁷Al magic-angle spinning NMR (MASNMR)⁵⁻¹², is well suited to answer these questions, because it provides direct information pertaining to the immediate local environment of two kinds of atoms. ²⁵Si MASNMR readily and quantitatively identifies the groupings Si(O-Al)_n(O-Si)_{4-n} where n = 4, 3, 2, 1 and 0; and ²⁷Al MASNMR easily distinguishes ^{13,14} between tetrahedrally and octahedrally bound Al³⁺ ions. (For brevity we write Si(nAl) for the grouping Si(O-Al)_n(O-Si)_{4-n}.)

It is proposed 15 that ultrastabilization proceeds as follows:

Thus the framework vacancies created by the removal of aluminium (accompanied by a decrease in ion-exchange capacity) are thought to be subsequently re-occupied by silicon ¹⁶ or aluminium ¹⁷. The interstitial aluminium can be removed by leaching with acid. Ultrastabilization is facilitated by the presence of water vapour. Materials with very high Si/Al ratios are prepared by repeated ammonium exchange, heat treatment and the removal of interstitial aluminium.

Despite detailed studies of stabilization using various techniques, its mechanism remains unclear, particularly concerning the origin of the silicon required for the proposed second stage. This may come from the surface or amorphous parts of the sample; or from extensive recrystallization. We shall demonstrate that a combination of ²⁹Si and ²⁷Al MASNMR provides new insights into the mechanism of ultrastabilization. In essence, by recording NMR spectra at the magic angle, extremely sharp resonance lines are obtained from solid samples, comparable in width with those normally obtained

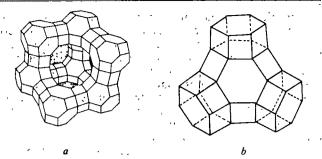


Fig. 1 Some features of the faujasite structure. a, The faujasite framework showing 14-hedral cages linked through six-membered rings, and supercages joined through circular windows. Tetrahedral atoms are located at the corners of polygons with oxygen atoms halfway between them. Non-framework cations are not shown. b, Tetrahedral linking of 14-hedral cages in faujasite. For clarity, the six-membered ring at the back of the cage is not shown.

from solutions or liquids. Chemical shifts are therefore measurable even in solids.

Experimental

²⁹Si MASNMR spectra were obtained at 79.80 MHz using a Bruker WH-400 narrow-bore spectrometer equipped with a magic-angle spinning attachment¹⁸ of the Andrew-Beams type¹⁹. Spinners, with an internal volume of ~450 μ l, were made of Delrin and spun at a rate of ~3 kHz. Cross-polarization and proton decoupling were not used. Intervals of 5 s were allowed between pulse sequences and 500–5,000 free induction decays were accumulated per sample. All ²⁹Si chemical shifts were measured from external tetramethylsilane, with high field shifts being negative. Experimental spectra have been computer simulated based on gaussian peak shapes, and the relative intensities of Si(nAl) signals calculated and corrected for the spinning sidebands. ²⁷Al MASNMR spectra were recorded at 104.22 MHz also using a home-made probe. ²⁷Al chemical shifts were recorded with respect to $[Al(H_2O)_6]^{3+}$ as an external reference; 3,000 free induction decays were accumulated per sample.

We have examined a series of four samples subjected to different types of treatment. Commercial zeolite Na-Y with Si/Al = 2.61, unit cell composition Na₅₃Al₅₃Si₁₃₉O₃₈₄·240 H₂O (established by X-ray fluorescence) and the (cubic) cell parameter a = 24.70 Å was the starting material. It was 75% exchanged with ammonium sulphate solution yielding NH₄-Na-Y zeolite (sample 1). Figure 2a shows the ²⁹Si MASNMR spectrum of this material; as expected, it is identical to that for the original Na-Y (not shown). Because in synthetic faujasites the first-order neighbourhood of every Al atom is Al(4Si), that is the Loewenstein rule strictly applies^{6,11}, the Si/Al ratio of the tetrahedral anionic framework can be calculated^{11,12} from the signal intensities I_{Si(nAl)} as

$$(\text{Si/Al})_{\text{NMR}} = \frac{\sum_{n=0}^{4} I_{\text{Si(nAl)}}}{\sum_{n=0}^{4} 0.25 n I_{\text{Si(nAl)}}}$$

thus providing a quantitative method of measuring zeolitic composition independently of elemental chemical analysis. Gaussian deconvolution of the ²⁹Si spectrum of sample 1 (Fig. 2a) leads to the ratio of intensities of the four peaks of 12.3:36.6:43.8:7.3, corresponding to (Si/Al)_{NMR} = 2.60, in excellent agreement with the value for the original Na-Y (by XRF and by NMR).

We note that when Si/Al ratios determined for synthetic faujasites by ²⁹Si MASNMR do not coincide with those obtained by chemical methods, it means that non-framework Al or Si: (such as detrital silica or adventitiously bound aluminous species) are present. This is clearly not the case in sample 1, the ²⁷Al spectrum of which is given in Fig. 3a. The single, relatively narrow signal with a chemical shift of 61.8 p.p.m.

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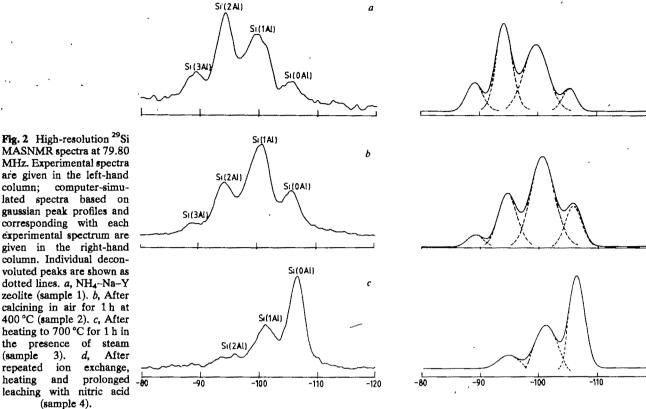
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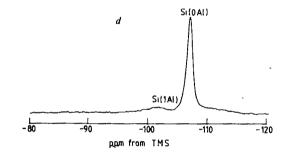
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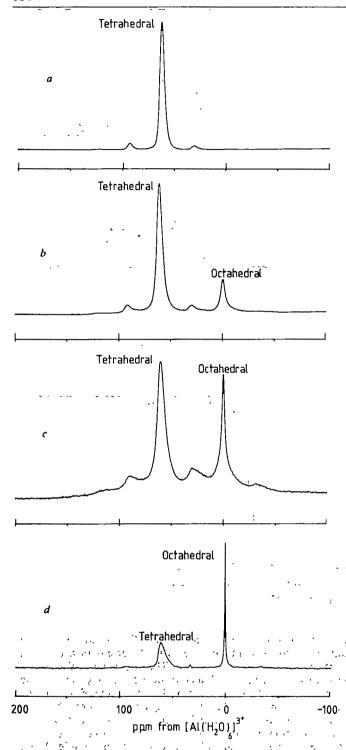
corresponds to the tetrahedrally bound aluminium (the small humps on the baseline, equidistant from the peak are spinning sidebands arising from chemical shift anisotropy).

Sample 2 was prepared by calcining sample 1 in air for 1 h at 400 °C. Its ²⁹Si spectrum (Fig. 2b) is significantly different from that for sample 1, showing a considerable decrease in the intensity of Si(3Al) and Si(2Al) signals with respect to Si(0Al). The calculated intensity ratio is now 4.8:24.6:55.3:15.3, leading to Si/Al ratio of 3.37 in the framework. Chemical analysis shows, however, no change in composition; clearly MASNMR is a superior analytical tool for the study of the status and quantity of chemical elements in the solid state. The observed increase in Si/Al ratio signifies that 23.3% of aluminium has been removed from the framework, with a corresponding loss of ion-exchange capacity. The ²⁷Al spectrum (Fig. 3b) is also markedly changed, showing two distinct signals: a large one (at 61.0 p.p.m.) corresponding to aluminium still remaining in tetrahedral sites, and a smaller one (at 0.59 p.p.m.) corresponding unmistakably to Al in octahedral coordination. This aluminium is now in the interstitial cationic positions, the ratio, r, of the intensities of the tetrahedral and the octahedral signals being 5.44 (the curve-fitted peaks were found to be exactly lorentzian). The total amount of aluminium in sample 2 is the same as in sample 1, as no washing was involved. Simple calculation leads to the expression Si/Al = 2.61(r+1)/r = 3.09for the composition of the tetrahedral framework. This value

is lower than 3.37 calculated from the ²⁹Si spectrum, but the difference corresponds to only three Al atoms in a unit cell containing 192 tetrahedral atoms. The intensities of the signals in both the ²⁹Si and ²⁷Al spectra do not display appreciable change over a 10-fold increase in the delay time of the experiment, which indicates that they are quantitatively reliable.

Sample 3 was prepared by heating NH₄-Na-Y to 700 °C for 1 h in the presence of steam. The ²⁹Si spectrum (Fig. 2c) is completely transformed with respect to that for samples 1 and 2. The Si(3Al) peak has disappeared, and the intensity ratio of the Si(2Al), Si(1Al) and Si(0Al) signals is now 10.7:36.7:52.6, leading to the framework composition $(Si/Al)_{NMR} = 6.89$. The Al spectrum (Fig. 3c) shows considerable amounts of octahedrally coordinated aluminium, as evidenced by the resonance at 0.0 p.p.m. There is also some amorphous material responsible for the raised baseline; it is therefore difficult to calculate the ratio of intensities reliably. We estimate r = 1.62, giving Si/Al = 4.20, lower than the value obtained from the ²⁹Si spectrum. The interstitial Al could be only partly octahedral; indeed, by assuming that one-third of it is tetrahedral, in accordance with the reported spinel structure for γ -alumina^{11,20}, we obtain agreement between the framework Si/Al ratios calculated from ²⁹Si and ²⁷Al MASNMR, respectively. The same holds for the small difference observed for sample 2.

Sample 4 was prepared by repeated application of the procedure used for preparing sample 3, followed by prolonged



²⁷Al MASNMR spectra at 104.22 MHz. a, Sample 1. Small humps equidistant from the peak are spinning sidebands. b, Sample 2. c, Sample 3. d, Sample 4.

leaching with 1 M nitric acid. The product has very low aluminium content, with Si/Al>50 (by chemical analysis) and a unit cell parameter a = 24.31 Å. The ²⁹Si spectrum (Fig. 2d) shows one sharp peak (FWHM = 1.1 p.p.m.) at -106.9 p.p.m.. and a very small broad signal at ~-101.3 p.p.m., clearly attributable to some residual Si(1Al) units. The sample contains amorphous material (note the slight elevation of the baseline), but its bulk is evidently very crystalline, the conclusion supported by the very sharp X-ray diffractogram and IR spectrum. The ²⁹Si MASNMR spectrum strongly suggests that framework vacancies created by the removal of aluminium are subsequently re-occupied by silicon. If this were not the case, the spectrum would be more complex, reflecting a range of possible environments for Si atoms including one, two or three neighbouring hydroxyl groups. Cross-polarization, involving Si-O-H bonds, would prove illuminating here. We note that the chemical shift of the Si(4Si) signal in sample 4 is very close to that reported for quartz⁵ (-107.4 p.p.m.).

The ²⁷Al MASNMR spectrum of sample 4 (Fig. 3d) contains a broad (FWHM=7.1 p.p.m.) tetrahedral peak at 61.0 p.p.m. and an extremely sharp (FWHM = 0.8 p.p.m.) octahedral signal at 0.23 p.p.m. The latter, the sharpest ²⁷Al signal we have measured in any solid, is due to motionally free [Al(H₂O)₆]³ in the cationic positions, not removed by leaching with acid. The ratio of intensities of the two signals is ~3. Such octahedral aluminium that still remains, neutralizes the residual negative framework charge caused by the presence of tetrahedrally coordinated aluminium. If all [Al(H₂O)₆]³⁺ ions are exchangeable, an ion-exchange equilibrium is established during acid leaching between [Al(H₂O)₆]³⁺ and H₃O⁺ competing for the cationic sites. Such equilibrium generally favours multivalent cations. (Note that one octahedral Al atom balances the charge of three tetrahedral Al atoms,)

Discussion

A recent study involving sorption measurements of gases²¹ showed that materials prepared in a manner similar to that for sample 4 contain a secondary mesopore system with pore radii in the range 15-19 Å, suggesting that the silicon which reoccupies empty tetrahedral sites does not come exclusively from the surface or from amorphous parts of the crystals, but also from their bulk, probably involving the elimination of the entire sodalite cages. MASNMR seems to support this conclusion. The Si(4Si) peak in Fig. 2d, although sharp, is almost twice the width of the analogous peak that we measured^{9,10} in the highly siliceous faujasite prepared by treating zeolite Y with gaseous SiCl₄, and of very similar aluminium content. This suggests that the structure of the thermally dealuminated material is less perfect than that of faujasite dealuminated using SiCl4.

We acknowledge support from the Universities of Cambridge and Guelph and from BP Research Centre, Sunbury. C.A.F. acknowledges the financial support of the Natural Sciences and Engineering Research Council (NSERC) of Canada in the form of an operating grant, G.C.G. the award of a NSERC graduate scholarship. The NMR spectra were obtained at the South-West Ontario High Field NMR Facility (Dr R. E. Lenkinski, Manager) funded by a Major Installation grant from NSERC.

Received 29 December 1981, accepted 2 February 1982

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Construction of a functional human suppressor tRNA gene: an approach to gene therapy for β -thalassaemia

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A human tRNA gene was converted to an amber suppressor by site-specific mutagenesis of the anticodon. The mutated $tRNA^{Lys}$ gene directed synthesis of a tRNA that suppressed the UAG amber nonsense mutation in β^0 thalassaemia mRNA. Such genes may be used to detect other nonsense mutations in mammalian cells and may provide an approach to gene therapy for β^0 thalassaemia due to nonsense mutations.

THE thalassaemias are a heterogeneous group of hereditary disorders characterized by defective globin chain synthesis1 They are broadly classified into two major subgroups, α and β thalassaemia, depending on whether α or β chain synthesis is impaired. Molecular studies reveal that many different kinds of mutations produce the thalassaemia phenotype. These lesions include structural gene deletions1, nucleotide substitutions in the intervening sequences that cause abnormal mRNA processing^{2,3}, and nonsense mutations in the coding region that lead to premature termination of globin chain production^{4,5}.

We previously characterized two different mutations that cause β^0 thalassaemia, a form of thalassaemia in which no β -globin chains are synthesized. In a Chinese patient, a AAG to UAG mutation converts the β^{17Lys} codon to an amber termination codon⁴, and in a Sardinian, a CAG to UAG mutation converts $\beta^{39\text{Gin}}$ to a terminator⁵. The latter mutation has been found in β^0 thalassaemia from other parts of Italy, Algeria and Morocco^{6,7}. The nonsense mutation in β^0 thalassaemia is suppressible in cell-free translation systems with a yeast ambersuppressor tRNA in vitro 5,8. If a functional suppressor tRNA gene were available, one could test its ability to overcome the nonsense mutation in β thalassaemia in vivo. We have now tested the feasibility of this approach by constructing a human suppressor tRNA gene and assessing its ability to suppress the nonsense mutation in β^0 thalassaemia in vivo in an oocyte

Transcription of human tRNA genes

A 1-kilobase fragment of human DNA that contains a tRNA Lys and a tRNA Gln gene has been isolated and completely sequenced (K. L. Roy, H. Cooke and R. Buckland, in preparation). The sequence of the tRNA^{Lys} gene is identical to the RNA sequence previously reported for rabbit tRNA^{Lys} (ref. 9). Neither gene contains intervening sequences. Based on their DNA sequences, tRNA transcribed from tRNA Lys and tRNA Gli should be 73 and 72 nucleotides long respectively, before the trinucleotide CCA is added to their 3' termini.

The sequence homology between the human tRNALys gene and the rabbit tRNA3 suggests, but does not prove, that the tRNALys gene is transcribed and the tRNA is functional. No information is available for the tRNA Gln gene. To test whether these two genes are transcribed in vivo, we injected them into the nuclei of Xenopus laevis, isolated the RNAs from the oocytes, and analysed the newly synthesized RNA on a polyacrylamide gel (Fig. 1a)^{10,11}. The major RNA products were 76 and 75 nucleotides long respectively, with some longer minor bands also visible. These findings suggested that the tRNA genes were transcribed and that the transcripts were processed.

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The lengths of the major tRNA bands indicated that CCA trinucleotides were added to the 3' termini of both genes. These bands were eluted from the gel, and their identities confirmed by fingerprinting (data not shown).

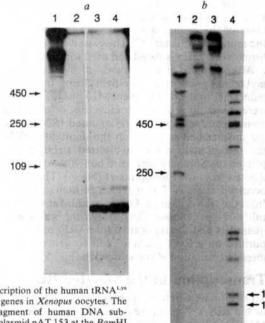


Fig. 1 Transcription of the human tRNA^{Lys} and tRNA^{Glo} genes in *Xenopus* oocytes. The 1-kilobase fragment of human DNA subcloned in the plasmid pAT 153 at the BamHI and HindIII site was cleaved with these two enzymes and EcoRI to produce the 220-bp and 800-bp fragments that contained the tRNA^{Gin} and tRNA^{Lys} genes respectively. Each fragment was subcloned in pBR322. An aliquot of 7 ng plasmid DNA (with either the tRNALys or tRNAGin gene insert), in a 25-nl solution that contained 10 mM HEPES (pH 7.9), 60 mM KCl, 0.3 μCi [α-32P]GTP (specific activity 450 mCi mmol⁻¹) was injected into the germinal vesicle of each oocyte 10,11. The oocytes were incubated at 19 °C in sterile modified Barth's solution (MBS-H) with 75 µg ml⁻¹ gentamycin and 40 µg ml⁻¹ bovine γ-globulin for 18 h. Each oocyte was

1 mg ml-1 Proteinase K, and incubated at 25 °C for 45 min. The RNA was twice extracted in phenol, precipitated in ethanol and electrophoresed on a 10% acrylamide gel containing 8 M urea¹². Autoradiography was performed for 3-18 h at -80 °C. Gel a, (1) DNA size markers (PM2 digested with HindIII); and RNA from oocytes injected with (2) pBR322, (3) and (4) pBR322 recombinants with the tRNAGIn and tRNALys genes respectively. Gel b, injection experiments similar to those shown in a performed with the RF of M13mp7 recombinants: (1) DNA size marker (PM2 digested with *Hin*dIII), (2) tRNA^{Lys} gene, (3) the mutated tRNA^{Lys} gene, (4) DNA size marker (M13mp7 digested with MspI).

then crushed and lysed in a 30-µl solution containing 100 mM Tris-HCl (pH 7.5), 300 mM NaCl, 10 mM EDTA, and

← 60

Mutation of the anticodon of the tRNA^{Lys} gene

We first mutated the anticodon region of the tRNA^{Lyn} gene to test its ability to function as an amber suppressor tRNA gene. The anti-sense strand of the tRNA^{Lyn} gene has the sequence AAA at the anticodon. To convert this gene so the tRNA will read the amber terminator UAG, we changed the AAA sequence to TAG using a synthetic pentadecamer (Bio Logicals) which was complementary to the anti-sense strand of the tRNA gene at the anticodon stem and loop, except for the two bases we intended to mutate. The sequences of the anticodon loop (anti-sense strand) of the tRNA gene and the primer used are shown in Fig. 2.

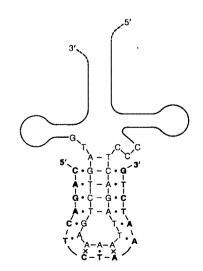
The strategy for mutagenesis is detailed in Fig. 3. The 800base pair (bp) DNA fragment that contained the tRNALy gene was subcloned into M13mp7 phage and single-stranded phage were isolated. The synthetic pentadecamer was used to prime synthesis of the complementary strand with DNA Polymerase I in the presence of T₄ DNA ligase in vitro, according to the method of Gillam and Smith^{17,18}. Initial synthesis was at 0 °C to maximize priming despite the mismatch; the temperature was then raised to 25 °C. The double-stranded closed circular forms were isolated on an agarose gel in the presence of ethidium bromide, and used to transfect Escherichia coli. Singlestranded phage were isolated from the culture and used as the template for a second round of synthesis which was carried out at 25 °C, without the initial 0 °C incubation, to favour hybridization of the primer with those phage that already contained the mutated sequence 18,19. The closed circular double-stranded forms were again isolated and used to transfect E. coli.

After a total of five rounds of synthesis, the success of mutagenesis was tested by hybridizing the ³²P-labelled pentadecamer to the recombinant M13 phage. Stringent conditions were used to allow hybridization of the primer to the homologous sequence in the mutated tRNA gene, but not to the mismatched sequence in the unmutated tRNA gene. All the recombinant phage hybridized strongly to the labelled primer on Southern analysis, but 90% of them had 300 bp deleted from the 800-bp insert (Fig. 4). The deletion probably occurred at an A-T rich region of human DNA downstream from the tRNA^{Lys} gene. One recombinant which contained the full 800-bp genomic DNA fragment was cultured in large quantities and the replicative form (RF) of DNA was isolated, purified on a CsCl gradient, and sequenced. It contained the intended mutation at the anticodon (Fig. 5).

Transcription of the mutated tRNA Lys gene

To determine whether the mutation affected the transcription of the tRNA^{Lys} gene, we injected the double-stranded RF of M13 DNA that contained the original and the mutated tRNA^{Lys} genes into the nuclei of *Xenopus* oocyte. Both produced

Fig. 2 Sequences of the anti-sense strand of the tRNA gene at the anticodon stem and loop, and the synthetic pentadecamer with the two-base pair mismatch (×) at the anticodon.



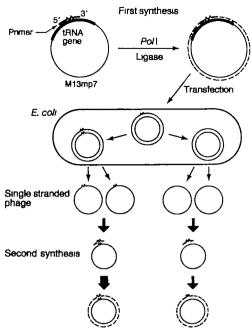


Fig. 3 Strategy of mutagenesis. The 800-bp DNA fragment that contained the tRNA^{L-n} gene with the BamHI and EcoRI ends was subcloned into the EcoRI site of the bacteriophage M13mp7¹³, after converting the BamHI site to an EcoRI site with linkers¹⁴. We selected the recombinant in which the anti-sense strand of the tRNA gene was contained in the single-stranded form of the M13 Single-stranded and RF forms of the recombinant phage were prepared 15.16. Site-specific mutagenesis with the pentadecamer was contained to Gullem and coworkers with modifications 17-19. A performed according to Gillam and coworkers with modifications¹⁷ 35-µl mixture that contained 2 pmol of the single-stranded M13 recombinant, 500 pmol of the synthetic pentadecamer, 100 mM NaCl, 40 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, and 2 mM β -mercaptoethanol was heated to 70 °C for 10 min and then cooled slowly over 1 h to 0 °C. A 28- μ l aliquot was removed and the mixture adjusted to a final volume of 70 µl, which contained 29 mM Tris HCl (pH 7.5), 40 mM NaCl, 14 mM MgCl₂, 1.4 mM β mercaptoethanol, 500 mM each of 4 dNTPs, 240 mM ATP, 90 U mi⁻¹ Klenow fragment of DNA Polymerase I (New England Biolabs) and 100 U ml⁻¹ of T₄ DNA ligase (Bethesda Research Laboratories). The muxture was incubated at 0 °C for 30 mm and then at 25-27 °C for 4 h. The DNA was then electrophoresed on a 0.8% agarose gel in the presence of 2 μg ml⁻¹ ethidium bromide. The band that contained the closed circular double-stranded DNA was excised and eluted with glass beads²⁰. This DNA was used to transform E. coli, strain GM48 (Dcm⁻, Dam⁻), previously made competent with CaCl₂ treatment. 0.3 ml of transformed GM48 cells was mixed with 1 ml of JM103¹³ (at A_{660} of 0.2), and an aliquot was plated to determine the titre and prepare individual plaques for screening. The remainder was grown overnight at 37 °C and singlestranded DNA isolated from the supernatant to serve as template for repeated in vitro synthesis. In subsequent rounds of synthesis, the conditions were modified to favour priming of the mutated species by decreasing the primer-to-template ratio to 25 to 1 and omitting the mitial incubation at 0 °C^{17,18}. A total of five rounds of synthesis was performed.

approximately the same amount of 76-bp tRNA products expected for tRNA^{Lys} (Fig. 1b). Thus mutagenesis did not alter transcription of the tRNA^{Lys} gene.

Ability of the mutated tRNA^{Lys} gene to direct suppression of the amber codon

We then tested the ability of the mutated tRNA^{1.79} gene to produce a functional amber suppressor tRNA. We used the mRNA from a patient with β^0 thalassaemia due to a $\beta^{17Lyn(AAG)\rightarrow UAG}$ mutation. The β/α globin mRNA ratio in this patient's reticulocytes is 0.15 (ref. 4). We previously showed that suppression with a yeast serine-inserting amber suppressor tRNA in a cell-free system produces a β -globin chain with an altered chromatographic elution pattern because the β^{17Lyn} is replaced with serine⁸. If the mutated human tRNA^{1.78} gene produced a functional amber suppressor tRNA and inserted lysine at the β^{17} position, we would expect that a β -globin chain with a normal charge would be produced. Also, the length of the β -globin chain would not be affected, since the normal termination codon of β -globin mRNA is UAA²⁵.

Xenopus oocyte nuclei were first injected either with the normal or the mutated tRNA^{Lya} gene. The cytoplasm of the

oocytes was injected 20 hours later with polyA⁺ reticulocyte RNA from the β^0 -thalassaemia patient. The oocytes were incubated for another 48 hours in the presence of ³H-histidine and the protein was extracted. The globin chains were immunoprecipitated using a monoclonal antibody, eluted, and analysed on a Triton X-urea gel²⁶. As expected, no β -globin synthesis was detected in the oocytes injected only with the mRNA, or with mRNA plus the normal tRNA^{Lys} gene. However, in the oocytes injected with the mutated tRNA^{Lys} gene, the β^0 thalassaemia mRNA now directed synthesis of an authentic β -globin chain, as identified by immunological reactivity and electrophoretic mobility (Fig. 6). Hence the tRNA^{Lys} gene with the anticodon mutation functioned as an amber suppressor and most likely inserted Lys into the β^{17} position of the β -globin chain.

Discussion

We previously showed that in the two known types of β^0 thalassaemia due to nonsense mutations, the UAG codon can be suppressed in vitro by adding a yeast suppressor tRNA5.8. We undertook the present study to see whether this finding could provide an approach for in vivo gene therapy. To conduct in vivo DNA transformation experiments, we needed a suppressor tRNA gene. Although suppressor tRNA genes have been derived from bacteria and yeast, evidence suggests that some yeast tRNA genes are not processed correctly in mammalian cells (P. Berg, personal communication). Mammalian tRNA genes were therefore preferable for our experiment. Suppressor tRNAs have been identified in rabbit reticulocytes2 and bovine liver²⁸, but since no mammalian suppressor tRNA genes have yet been isolated, we constructed one in vitro. We mutated the anticodon of a tRNALys gene (AAA) to TAG (anti-sense strand) with a primer mismatched at the two-base sequence. We showed that this gene was transcribed and that the transcript functioned as an amber suppressor in vivo in Xenopus oocyte, because it read through the UAG codon in β^0 -thalassaemia mRNA. It is thus possible to convert a tRNA^{Lys} to an amber suppressor tRNA by mutating the anticodon.

The availability of mammalian suppressor tRNA genes offers a means of detecting nonsense mutations in higher organisms. At present, apart from viruses, bacteria and yeast, the only naturally occurring nonsense mutations known to cause diseases in higher organisms are the two defined in β -thalassaemia in humans⁴⁻⁶. Other nonsense mutations undoubtedly occur; just in the coding region of the β -globin gene there are 29 positions at which a single nucleotide substitution could produce a termination codon. Certain mouse and Chinese hamster cell lines with hypoxanthine guanine phosphoribosyl transferase

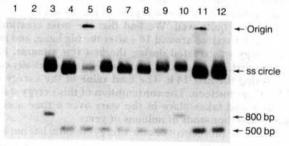


Fig. 4 Southern analysis of M13 recombinant phage. After the fifth round of synthesis, RF DNA from the cell pellet 16 was prepared from individual plaques, and the RF DNA from 35 of the mutated clones was digested with *Eco*RI, electrophoresed on an 0.8% agarose gel and transferred to nitrocellulose filters 22. The filters were prehybridized for 2 h, and then hybridized for 16 h with the synthetic pentadecamer, which was previously labelled with 32P at the 5' end, in a buffer containing 6×SSC, 10×Denhardt's solution at 41°C and 20 μg ml-1 yeast RNA. The filter was washed three times with 6×SSC for 20 min at 12°C23, dried and autoradiographed. *Lane 1*, DNA from M13; *lane 2*, M13 containing the unmutated tRNA¹⁻³⁷ gene; *lanes 3-12*, ten of the DNA samples containing the M13 tRNA¹⁻³⁷ gene after mutagenesis. All 35 clones isolated hybridized strongly to the probe. In all but two of the clones shown, deletions shortened the insert from 800 to 500 bp. The DNA from the clone shown in lane 10 was grown in large volume for further studies.

Fig. 5 Sequencing gel showing the mutated sequence in the anticodon region of the tRNA^{1,58} gene. The RF DNA prepared from the mutated clone shown in Fig. 4 was purified by CsCl banding for 48 h. A KpnI site, 55 bp from the anticodon was labelled with ³²P at the 3' terminus using T₄ DNA polymerase²⁴, and the sequence of the tRNA gene was determined by the method of Maxam and Gilbert on a 15% polyacrylamide gel in 8 M Urea¹².

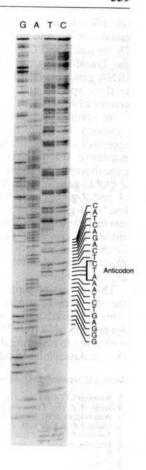
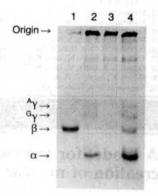


Fig. 6 Analysis of globin chain synthesis in Xenopus oocyte after injection with tRNA genes polyA+ mRNA from human reticulocytes and immunoprecipitation of globin with monoclonal antibodies. A sample of 7 ng DNA that contained either the normal or mutated tRNALys gene was injected into the nucleus of each Xenopus oocyte as described in Fig. 1, but without [α-32P]GTP. 20 h later, 10 ng of polyA+ reticulocyte RNA in 50 nl of 0.01 M Tris-HCI (pH 7.5), 0.02 M KCl, and 0.25 mM haemin injected into the cytoplasm at the equatorial band and incubation was continued at 19°C for 48 h after



adding 0.2 mCi ³H-histidine to each ml of the medium. Oocytes were washed twice in the incubation buffer, then squashed in a 5-µl solution per oocyte of 100 mM Tris-HCl (pH 6.8), 150 µg ml⁻¹ phenyl methyl sulphonyl fluoride (PMSF), and 2.5 mM EDTA. The lysate was clarified twice by centrifugation at 10,000 r.p.m. in an Eppendorf centrifuge. The extract from three oocytes was pooled and reacted with 25 µg of a monoclonal antibody against human globin in a 50 µl TENT buffer (25 mM Tris [pH 7.4], 10 mM EDTA, 350 mM NaCl, 0.15% Triton X-100, 1 mg ml⁻¹ bovine serum albumin). (This antibody reacts most strongly with human β -chains, but also reacts with α- and γ-globin chains.) After 18 h at 4 °C, 50 μl of a 10% suspension of Staphylococcus A in TENT buffer was added, and incubated at 4 °C for 6 h. The Stophylococcus A was pelleted by centrifugation at 10,000 r.p.m. for 2 min at 4 °C, and washed three times with the TENT buffer (without bovine serum albumin). Haemoglobin A 1 µg was added as carrier and the bound radioactive globin was eluted by heating the pellet to 90 °C for 2 min in a 25-μl buffer containing 6 M urea (BioRad, electrophoretic purity), 1 M 2-mercaptoethanol (Sigma), 2% Triton X-100, 0.33 mg ml⁻¹ Pyronin-Y. The cells were pelleted and the supernatant applied to a Triton X urea gel The gels were dried, soaked in Enhance (New England Nuclear) and autoradiographed for 4 days. Lane 1, oocytes injected with normal β -globin mRNA; lane 2, injected with β^0 mRNA alone; lane 3, injected with the mutated tRNA^{1.ys} gene only; lane 4, injected with β^0 mRNA and the mutated tRNA^{1.ys} gene. When the β^0 mRNA was injected together with the original tRNA^{1,38} gene, the result was identical to that shown in lane 2. The migration of the α -, β -, α -, and α -globin chains was determined with unlabelled globin markers.

(HGPRT) deficiency are also suspected to be due to nonsense mutations^{29,30}, and nonsense mutations have been detected in Drosophila³¹ and Caenorhabditis elegans^{32,33} by genetic analysis. Transforming cells from such organisms with suppressor tRNA genes may confirm the presence of nonsense mutations in these species and detect such mutations in other human genetic disorders.

Insertion of suppressor tRNA genes can be tested as an approach to gene therapy in β -thalassaemia. While this approach would be limited to β -thalassaemias due to nonsense mutations, it has a theoretical advantage over other proposed gene therapies for β -thalassaemia, such as insertion of a normal β -globin gene into thalassaemia cells. In experiments where a cloned β -globin gene was inserted into heterologous cells, the level of β -globin expression was low³⁴. At present, the mechanism that controls normal β -globin gene expression is not understood. In contrast, since tRNAs are essential for protein synthesis in all cells, inserted functional tRNA genes are likely to be expressed, even in differentiated cells such as erythroid cells.

The success of suppressor tRNA gene therapy depends on the efficiency of suppression by the mutated tRNA. If, as in some bacterial and yeast suppressor tRNAs, the efficiency approaches 60% (refs 35, 36), then the gene should significantly contribute to restoring the balance of α - and β -globin synthesis in β -thalassaemia. In addition, the efficiency of suppression of

Received 15 September 1981; accepted 23 February 1982

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suppressor tRNAs can vary. It is therefore important to test the effects of multiplicity of gene insertion and the degree of suppression with various tRNAs. We are currently mutating the tRNA^{Gln} gene in a similar manner in order to study the Mediterranean type of β -thalassaemia due to a β ^{39Gln(CAG) \rightarrow UAG} mutation⁵.

Of course, before the use of suppressor tRNAs can be contemplated for gene therapy, their effect on other cell functions must be assessed. An amber-suppressor tRNA could also suppress termination of proteins that utilize the UAG codon as their normal terminator. Although suppressor tRNAs are lethal in certain yeast strains, no information is available on their effects on cells of higher organisms. The availability of a functional suppressor tRNA gene allows us to test for any deleterious effects it may have on mammalian cells.

We thank Dr Rae Lvn Burke for suggesting the use of an ethidium bromide agarose gel to separate the different forms of synthetic M13 products, Drs Michael Smith and U. L. RajBhandary for their advice on mutagenesis, Dr George Stamatoyannopoulos for the antibody against human globin, and Dr Joe Hedgepeth for the GM48 strain of E. coli and J. Gampell for editorial comments. This research was supported by grants from NIH (AM 16666), the March of Dimes/Birth Defects Foundation, and the Natural Sciences and Engineering Research Council of Canada. Y.W.K. is an investigator of the Howard Hughes Medical Institute.

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A model for the cosmic creation of nuclear exergy

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The exergy of a physical system is the maximum amount of mechanical work that can be extracted from that system so that a system in thermodynamic equilibrium has zero exergy. An old theme in cosmic thermodynamics is that the second law implies that the Universe is running down (running out of exergy) and approaching thermodynamic equilibrium, 'heat death'. However, in the standard model of the early Universe2, at 0.01 s after the big bang the Universe consisted of ordinary matter (nucleons), electrons and positrons, neutrinos, photons and gravitons. The gravitons were decoupled, but the others were in thermodyamic equilibrium. Thus the Universe had already reached the state of heat death, and its exergy was zero. The main problem is, therefore, not to describe the runningdown of the Universe, but to understand its revival. When and how was exergy created, in particular the nuclear exergy, which is transformed into life-supporting light in our Sun? We have studied a model which should represent the nucleon gas of the early Universe quite well. We find that the main creation of nuclear exergy started around 10 s after the big bang, and most of the exergy was created during the first few minutes, 85% during the first hour, and that the process was essentially completed during the first 24 h. The final value of the exergy was 7.72 MeV per nucleon. The consumption of this exergy started much later and takes place in the stars over a time scale of hundreds and thousands of millions of years.

Although the problem of cosmic exergy creation has not been widely discussed, general answers have been given by Tolman³, and later by Layzer⁴ and Landsberg⁵. The solution is that during the expansion of the Universe, the conditions for local equilibrium changed with changing temperature and pressure. The reactions tending towards equilibrium, however, lagged behind, and disequilibrium arose. This does not violate the second law of thermodynamics. If one considers any element $\Delta\Omega$ of the Universe, its entropy ΔS increases, but its maximum attainable entropy ΔS_{max} may increase faster due to the expansion, which thus induces a growing disequilibrium.

Work can be extracted from a system only to the extent that this deviates from equilibrium. Exergy is thus a measure of deviation from equilibrium, of contrast, internally or against

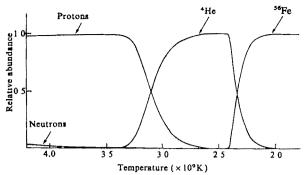


Fig. 1 Relative abundances $P_i^{(0)}$ at equilibrium for the four nucleon states considered in the nucleon gas model.

an external background^{1,6-9}. For instance, in an environment of temperature T_0 , a package of heat ΔQ at temperature T has the exergy

 $\Delta E = \Delta Q \left(1 - \frac{T_0}{T} \right) \tag{1}$

that is here exergy equals energy times a quality factor, which is simply the Carnot factor $1-T_0/T$. As the quality may decrease (with decreasing contrast), unlike energy, exergy is not conserved. Physical processes always go towards increasing entropy which usually means that systems move towards equilibrium and thus towards decreasing exergy. Therefore to attain a state away from equilibrium or to sustain such a state, an exergy input is required.

The totally dominant exergy source of the Earth is the inflow of solar radiation, the value of which derives from its contrast with the ambience of the Solar System, the cold interstellar space. The solar radiation derives from a continuous conversion of nuclear exergy in the interior of the Sun. Our problem is to understand how this exergy arose through the mentioned mechanism. In the nucleon gas of the early Universe, exergy and entropy increased together, and we shall study a simple statistical model for this process.

The era we are considering is dominated by radiation, and the photon gas may be considered as a heat bath which is continuously cooling down. The nucleon gas is always in thermal contact with this bath. Below a certain temperature $(2.0 \times 10^9 \text{ K})$ an iron nucleus (^{56}Fe)—if it could be formed by a coincidence of events (extremely unlikely) or be introduced from outside—would be thermally stable in contact with the heat bath. From then on the nucleon gas may be considered as being overheated, the natural phase at this temperature being a gas of iron nuclei. (The overheating started much earlier, first through an overabundance of neutrons, which decay only slowly. Also for a short period at temperatures near $2.5 \times 10^9 \text{ K}$ (well before helium synthesis) ^4He defines the natural phase of the nucleon gas.)

Several bottlenecks prevent the formation of iron nuclei, the first being the low binding energy of the deuteron. After ⁴He has been formed (through ²H, ³H and ³He) at a temperature below the dissociation temperature of deuterons, density and temperature are already below the values required to sustain continued nuclear fusion reactions beyond helium.

The exergy is thus created, when the nucleon gas makes the transition from the equilibrium state to the overheated state. Some exergy is consumed in neutron decay and in helium formation. In the latter practically all remaining neutrons are absorbed. Helium formation is a relatively rapid process, and in our model it occurs at a sharp instant of time.

In the era under consideration the temperature is still high and the density is sufficiently low to consider the nucleon gas as a non-relativistic Maxwell–Boltzmann gas. Consider again an element $\Delta\Omega$ of the gas. The exergy 10,11 per nucleon is

$$E = kT \sum_{i} P_{i} \ln \frac{P_{i}}{P_{i}^{(0)}}$$
 (2)

where i labels states and where P_i is the actual probability

distribution for one nucleon, and $P_1^{(0)}$ is the probability distribution for one nucleon at thermodynamic equilibrium.

We shall consider a simplified model. As helium forms early in the history of the Universe, and because iron is an end product of nuclear burning, we shall include the ground states of 4 He and 56 Fe in our model but no other composite nuclei. Thus a nucleon may either be a free proton or a free neutron or it may be bound in 4 He or 56 Fe. We shall label these possibilities by an index i of value 1-4.

At low temperatures the statistical factors depend sensitively on the energy levels. This tends to lead to an all-or-none situation. Below the temperature 2×10^9 K, 56 Fe totally fixes the normalization of the $P_i^{(0)}$, and inclusion of other nuclides with tighter binding than 4 He would not change the general picture. Thus it is sufficient to include in equation (2) only 56 Fe and states that are actually occupied. From those latter states we have excluded the transitional nuclides, 2 H, 3 H, 3 He, which are important for the detailed dynamics, but which, due to their relatively low abundance, would change E as a function of temperature only marginally.

At temperature T the equilibrium distribution of the nucleons over the four nuclear states is

$$P_{i}^{(0)} = \kappa r \left(\frac{mc^{2}}{kT}\right)^{3/2} g_{i} A_{i}^{5/2} \exp\left[-A_{i}(\mu(T) - b_{i})/kT\right]$$
 (3)

where g_i is the spin multiplicity of states, A_i is the mass number of the nuclide, and b_i is the average binding energy per nucleon (relative to a free proton); m is the nucleon mass, r is the ratio of photon to nucleon abundance in the Universe, κ is a constant, and $\mu(T)$ is the chemical potential.

Table 1 gives A_n g_i and b_i and further

$$\begin{cases} m = 938.8 & \text{MeV/}c^2 \\ \kappa = \frac{\sqrt{2\pi}}{8\zeta(3)} = 0.351 \end{cases}$$
 (4)

The quantity r is not very well known¹²⁻¹⁴. We shall use the value

$$r = 10^9 \tag{6}$$

The results of our calculation do not depend very strongly on the exact value chosen for this parameter.

The chemical potential $\mu(T)$ in equation (3) is fixed by the overall normalization condition

$$\sum_{i} P_{i}^{(0)} = 1 \tag{7}$$

The derivation of equation (3) follows standard methods of statistical mechanics.

The actual probability distribution is given in Table 2. It changes abruptly when helium is formed, which in our model takes place instantaneously at the temperature

$$T_0 = 1.0 \times 10^9 \,\mathrm{K} \tag{8}$$

The picture would change only marginally if T_0 were changed, and the asymptotic exergy value (7.72 MeV per nucleon) depends on T_0 only through $\gamma(T_0) = \gamma_0$, where $2\gamma_0$ is the fraction of primordial helium, for which we use the value 27% (ref. 2).

In general the neutron fraction is a complicated function, which has to be computed numerically 15-17. The same is true of the relation between temperature and time.

Table 1	Characteristics of the four nuclides considered					
ı	A_iZ_i	g,	b, (MeV)			
1	¹ H .	2	0			
2	¹ n	2	-1.30			
3	⁴He	1	6.43			
4	⁵⁶ Fe	1	8.10			

Table 2 Actual distribution of nucleons over the four nuclides before and after helium formation

								`
	i				$P_{\iota}(T > T_0)$			$P_{i}(T < T_{0})$
,	1	-		-	$1-\gamma(T)$			$1-2\gamma(T_0)$
	2	,	, ,		$\gamma(T)$		•	· 0 ·
	3 ·				0	•		$2\gamma(T_0)$
	4 .	٠.			0			i 0

 $\gamma(T)$ is the neutron fraction.

The exergy per nucleon from equation (2) is now obtained from equation (3) and Table 2,

$$\begin{cases}
E(T) = \mu(T) + |b_2|\gamma(T) \\
-kT \ln \frac{2\kappa r \left(\frac{mc^2}{kT}\right)^{3/2}}{(\gamma(T))^{\gamma(T)}(1 - \gamma(T))^{1 - \gamma(T)}}; & T > T_0 \\
E(T) = (1 + 6\gamma_0)\mu(T) - 8\gamma_0 b_3 \\
-kT \ln \frac{2^{8\gamma_0 - 1}\kappa r \left(\frac{mc^2}{kT}\right)^{3/2}}{\gamma_0^{\gamma_0}(1 - \gamma_0)^{1 - \gamma_0}}; & T < T_0
\end{cases} (10)$$

The equilibrium probability distribution (3) is plotted in Fig. 1. The nucleons dominate completely for $T > 3.5 \times 10^9$ K, and iron dominates for $T < 2 \times 10^9$ K. Helium dominates in an interval around 2.5×10^9 K.

The creation of exergy is shown in Fig. 2. The exergy loss in helium synthesis is ~0.6 MeV. Part of this is compensated for in the following evolution, and the net loss is ~ 0.4 MeV. The final value is given by equation (10) in the low temperature limit,

$$E(0) = (1 + 6\gamma_0)b_4 - 8\gamma_0b_3 = 7.72$$
 MeV (11)

The nuclear burning in stars with iron as an end product takes place relatively late, and several barriers have to be overcome¹⁸. Only a limited fraction of the nucleons in a star are involved in these reactions. Due to the cold environment the relative exergy loss in the transition from nuclear to radiation exergy in a star is small, of the order of 1% or fractions thereof.

At one stage in the expansion of the Universe matter became so disperse that the thermal contact with surrounding matter

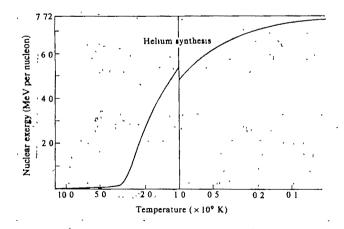


Fig. 2 Nuclear exergy as a function of the cosmic temperature T. Helium synthesis in this model takes place instantaneously at $T = 1.0 \times 10^9$ K. It involves an exergy consumption of around 0.6 MeV per nucleon, one-third of which is compensated for by the subsequent exergy production. This can be compared with the exergy loss of around 10 eV per nucleon some 700,000 yr later, when the electrons condense into atoms.

and radiation could no longer prevent density fluctuations from being enhanced by gravitational attraction. Then a new feature entered the picture. When this happened, matter already dominated over radiation in the Universe. The enhancement of density fluctuations meant that the Universe split on several levels into 'compartments' (protogalaxies and protostars) with relatively little interaction with the exterior. Within each compartment the natural reference state is the equilibrium end state for that compartment. Thus at the time of compartmentalization new exergy was created. For a protostar which is to end up as a white dwarf the nuclear exergy from the first few minutes is augmented by some gravitational exergy (of the order of a few keV per nucleon).

For a more massive protostar which is to end up as a cold neutron star there is also a substantial addition of combined nuclear-gravitational exergy of the order of 40 MeV per nucleon¹⁹. The gravitational exergy here gives the dominant contribution. The heavy elements (heavier than iron) formed in a supernova explosion with a neutron star as the final state for its core, thus have exergy which is mainly of this origin.

For a system large enough to have a black hole as its final state, the exergy per nucleon may be a large fraction of its rest energy20

If matter in the form of nucleons is unstable, as modern elementary particle physics suggests²¹, then matter itself represents a special form of exergy, which was created during the first few microseconds, when an excess of matter over antimatter was developed followed by annihilation of all antimatter and most of the matter²²⁻³¹. Matter as an exergy form is extremely longlived but if the Universe is open and expands for infinity then this exergy will also finally be consumed in nucleon decay (with a half life of $\sim 10^{31}$ yr) and at the end the Universe will consist of ever cooler electrons and positrons (too disperse to annihilate completely), neutrinos, photons and gravitons.

We thank Göran Niklasson and Ture Eriksson for clarifying discussions on the properties of the nuclear gas and on relativistic cosmology. B.-S.S. also thanks J. Prentki and the CERN Theory Division for hospitality. This work was supported by the Swedish Council for Planning and Coordination of Research.

Received 5 October 1981, accepted 3 February 1982

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Cygnus X-3 observed at photon energies above 500 GeV

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We have used the twin 11-m diameter mirrors of the NASA Jet Propulsion Laboratory's solar energy facility to observe highenergy γ rays from Cyg X-3 using the atmospheric Cerenkov technique. Observations of photons with energies above 10¹² eV modulated with the 4.8 h period of the X-ray source have been reported previously by the Crimean Astrophysical Observatory¹⁻⁶ and the Fred Whipple Observatory^{7,8}. We report here data from ~105 air shower events obtained 29 August-6 September 1981 with an approximate threshold energy of 500 GeV. A positive signal with an amplitude of 10.9 ± 2.5% of the background cosmic ray rate appears near phase 0.6 of the 4.8-h cycle, in which phase 0.0 corresponds to minimum X-ray emission. This observation, together with previous Cerenkov detections, indicates that the high energy emission from Cyg X-3 is evolving on a time scale of a few years which suggests a recent origin for the system in its present form.

The JPL solar energy mirrors are located at Edwards Air Force Base, California at a dry desert location at an elevation of 0.7 km. Each mirror was viewed by a single, fast photomultiplier which gave full fields of view of 1.0° and 1.8°. (Requirements of compatibility with the solar research dictated the unequal size tubes.) The mirrors are separated by 24 m. The rate of air shower coincidences between the two mirrors is ~60% of the rate for a single mirror. Preliminary tests with the mirrors in June–July 1981 gave a background coincidence rate of ~4 s⁻¹ for air shower events near the zenith. However, before our August–September run, the focus of one of the mirrors was changed for solar energy purposes to reduce the effective size of the mirror to ~5 m. The data reported here were obtained in conditions in which the background rate at the zenith was about 1 s⁻¹.

Extensive alignment tests and angular calibrations were performed with each mirror using the image information from paraxially mounted television cameras. During observations the pointing of each mirror was monitored and found to be reproducible to better than 0.1°. Data associated with air shower events were recorded magnetically when signals from the two detectors occurred within 50 ns of each other. The pulse height associated with each detector, the time difference between the two signals, and the universal time to 0.1 ms were recorded. In addition singles and accidentals rates were monitored. The accidental rate for most scans was <1%. Analysis of the data offline showed that coincident events were characterized by a peak whose FWHM was ~10 ns, equivalent to a resolving time of ~5 ns. For the data reported here we have selected events with a 'tight' coincidence requirement corresponding to a resolving time of 3.8 ns.

Observations were made in drift scans which typically lasted 33 min, starting 17 min before and ending 16 min after transit. Following the procedure of Danaher $et\,al.^7$, we divided the data from an individual drift scan into three regions: two background regions more than 5 min before and after source transit, and an on-source region. We used an on-source region of 7 min centred at the source transit time. From a sum of the drift scans we determined that the 2nd mag star, γ Cygni, which transited 10 min before Cyg X-3, increased the background rate by $3\pm$

1%; therefore we have deleted data during an interval of 6 min about γ Cyg from our analysis.

The data rate was analysed in 30-s bins. A scan was rejected if: (1) any 30 s interval gave a $\pm 4\sigma$ departure from the mean; (2) the two background observations failed to agree at the 5% level; (3) a χ^2 uniformity test for the run as a whole failed at the 5% level. In addition a Poisson homogeneity test at the 5% level was applied to the data binned in 10 s bins. Of 95 drift scans recorded during the 9 day run, 81 passed all tests.

Figure 1 shows the ratio of the on-source counting rate to the background counting rate corresponding to various phases of the 4.8-h periodicity. Errors have been calculated both experimentally and on the basis of Poisson statistics. For the scans which passed the selection tests there is no significant difference, and the Poisson values are used. For our phase calculation we have used the X-ray parameters of van der Klis and Bonnet-Bidaud⁹. Their quadratic and linear parameters give a difference in phase of 0.04 at our epoch. We have used the quadratic parameters tied to their well determined $T_{\rm min}$ of May-June 1980. If the Crimean values are used then the phases of Fig. 1 are increased by 0.115.

Eight of the 10 phase bins are consistent with no signal. However, in the adjacent phase bins from 0.5 to 0.7 there are 3.1 and 2.9σ excesses, which together constitute a 4.2σ excess which we interpret as a positive detection. If we bin the data in the signal region in bins of 0.05 phase, the ratios and significances for the four bins beginning at 0.5 phase are: 1.04 (0.7σ) , 1.12 (3.2σ) , 1.09 (2.2σ) , and 1.08 (1.9σ) . The 23 scans corresponding to phases between 0.5 and 0.7 have been added together and their sum is shown in Fig. 2a. These data have been fitted to a level background plus a gaussian of variable width and height centred at the transit time. The best fit value of the height is $10.9 \pm 2.5\%$ of the background, equivalent to a 4.4 σ detection. Figure 2b shows the sum of the scans with phases outside of the range 0.5-0.7. The horizontal line corresponds to the average background rate. The flux averaged over a cycle corresponding to the effect is $\sim 8 \times 10^{-11}$ photons cm⁻² s⁻¹ in reasonable agreement with previous detections at higher energies.

The phase of the observed emission, 0.6 ± 0.1 , agrees with the phase typically seen for maximum X-ray emission⁹ and agrees approximately with the 1980 observation of Danaher et al.⁷, after accounting for the different methods of phase calcula-

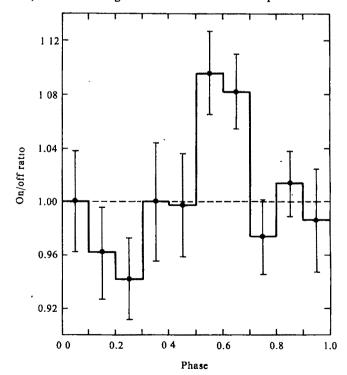


Fig. 1 Phase histogram of the γ -ray emission from Cyg X-3.

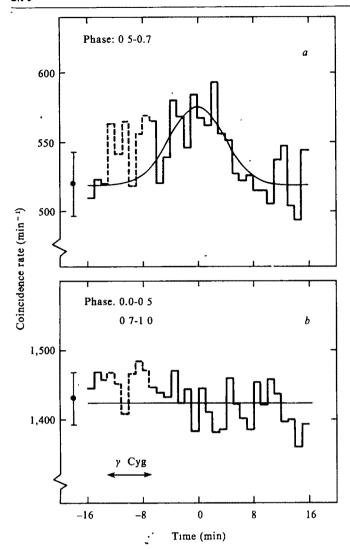


Fig. 2 Coincidence rate in c.p.m. versus drift scan time. The transit of Cyg X-3 occurs at time 0. a, The sum of the 23 scans corresponding to phases between 0.5 and 0.7. The smooth curve is the best fit of a gaussian, centred at the transit, plus a level background. The height of the gaussian is $10.9 \pm 2.5\%$ of the background, equivalent to a 4.4σ detection. b, The sum of the remaining scans. The horizontal line corresponds to the average background rate. For these phases there is no evidence for γ -ray emission from Cyg X-3. The dotted portion of each histogram corresponds to the 6-min interval in which the 2nd mag star, y Cyg, is in the field of view. These data were not used in any of the analyses.

tion⁸. However, the width of ~0.2 cycles seen here may be inconsistent with their results and is inconsistent with the Crimean results which have been interpreted as indicating activity confined to 0.05 cycles^{4,6}. Also, we see no evidence for emission at other phases as observed in some of the earlier Crimean measurements. The disparity in these observations may indicate changes in the characteristics of the high energy emission on a time scale of a few years. These apparent changes and the apparent 100 MeV variability implied by the SAS 2 detection 10 and the COS B upper limit 11 support the view that Cyg X-3 is a system which is rapidly evolving, and that it may have been created in its present form in the recent past. As has been pointed out 12,13, the presence of high energy γ rays favour models with a young pulsar in rapid rotation. If the pulsed fraction increases with energy as in the case of the Crab¹⁴ and Vela¹⁵ pulsars, then future high statistics ground based measurements may be the most promising method of detection.

We thank the staff of JPL Edwards Test Site for cooperation and especially John Woodbury, Terry Hagen, and Jack Whitehead. W.A.W. was supported by an NRC Resident Associateship during part of this work. This work was supported partially by the US Department of Energy, NASA, and a grant from the California Institute of Technology President's Fund.

Received 11 January; accepted 19 February 1982

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Characterization of radioactive fallout from pre- and postmoratorium tests to polar ice caps

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Polar glaciers contain a detailed historical record of artificial radioactive fallout from the atmosphere1-4. The records are continuous and time frames can be introduced with uncertainties of about ±1 yr. We have previously indicated that there may be characteristic nuclide compositions of fallout produced by weapons testing by different countries from records in ice sheets. For example, the testing period dominated by the US activity in the early 1950s produced a markedly low ²³⁸Pu/²³⁹⁺²⁴⁰Pu ratio and a very high ²⁴¹Pu/²³⁹⁺²⁴⁰Pu ratio compared with those resulting from tests dominated the USSR activity in the early 1960s^{1,3,5}. Here we use analyses on a recently collected Greenland ice sheet core to extend these results and find the characteristic ²³⁹⁺²⁴⁰Pu/¹³⁷Cs and ²³⁹⁺²⁴⁰Pu/⁹⁰Sr ratios for these two periods. The atmospheric behaviours of tritium compared with those of other artificial radionuclides are recorded in its fluxes to the polar ice sheets.

From 40-m deep, 7.5-cm diameter firn cores were recovered from Dye-3 (65°11' N, 43°50' W) in June 1980 as part of the joint US-Danish-Swiss Greenland Ice Sheet Program. Density measurements and visible stratigraphical observations were made on each core section. The cores were then stratigraphically correlated and combined to form individual samples for transuranic and fission product analyses representing 2 yr on average. ³H analyses were performed on the yearly samples. The analytical techniques have been described elsewhere¹.

The average accumulation rate, based on ²¹⁰Pb profile in the glacier, is 52 g H₂O cm⁻² yr⁻¹ (Fig. 1a). The inventory of ²¹⁰Pb, corrected for decay to the time of collection, to the Dye-3 site (9.8 mCi km⁻² for the period 1944-80) is similar to that of South Dome, Greenland (63°31.6' N, 44°34.5' W), for the period 1945-75 that we measured previously (10.2 mCi km⁻ ref. 1). A lower ²¹⁰Pb inventory of 4.4 mCi km⁻² is computed

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for the Crete, Greenland, Site at 71°07′ N and 37°19′ W (ref. 6). The ²¹⁰Pb derived accumulation rate compares well with the long-term average rate of 49 g cm⁻² yr⁻¹ determined from stable oxygen isotope analysis⁷. The samples cover on average two year intervals with a range of 1–3 yr, based on the ²¹⁰Pb ages (Fig. 1).

The plutonium inventories to the northern and southern hemispheric polar sites indicate a higher contribution from the US dominated pre-moratorium atmospheric nuclear tests compared with the USSR dominated post-moratorium atmospheric nuclear tests (Fig. 1; Table 1) than previously estimated. The moratorium period was from November 1958 to September 1961. On the basis of the total atmospheric bomb yields of these two periods, the ratio⁸ of post- to pre-moratorium testing is around 70:30. On the basis of the accumulated amounts of

plutonium in both Arctic and Antarctic ice sheets, the contributions from the pre-moratorium tests varies between 41 and 64%. The value from three of the four sites clusters about 43%.

On the other hand, the ¹³⁷Cs and ⁹⁰Sr results (Table 1) accord with the 70:30 ratio from the bomb yields. This was expected because ¹³⁷Cs and ⁹⁰Sr are fission products whereas Pu is not. Pu production does not necessarily correlate with total bomb production as it is also dependent on the type of weapon construction.

The integrated fluxes of ²³⁸Pu and ²³⁹⁺²⁴⁰Pu are about a factor of two lower in the Dye-3 Greenland sites compared with those of the South Dome Greenland site. The fluxes are: ²³⁸Pu, 0.003 and 0.006 mCi km⁻²; and ²³⁹⁺²⁴⁰Pu, 0.27 and 0.7 mCi km⁻². This situation may arise from unique meteorologies associated with the delivery of stratospheric fallout to the two areas, only

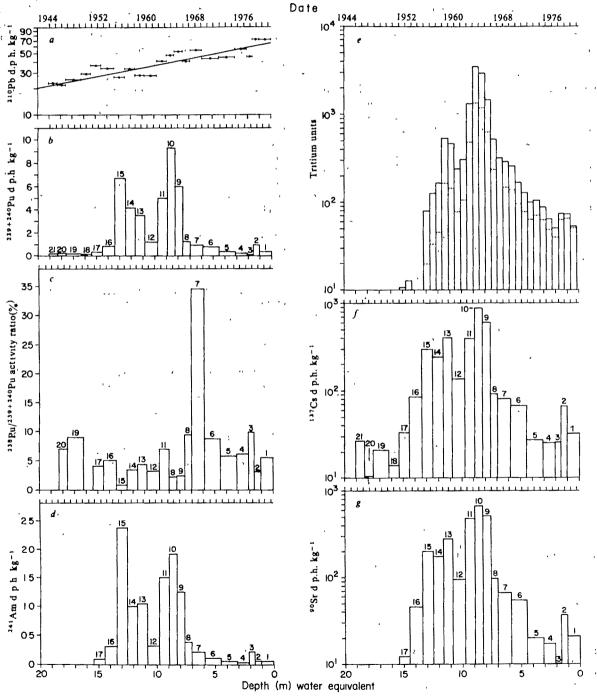


Fig. 1 Radionuclides in the Greenland Ice Sheet at Dye-3 Station. The time scale is derived from the ²¹⁰Pb profile (a). The ²³⁸Pu (c), ³H (e), ¹³⁷Cs (f) and ⁹⁰Sr (g) concentrations are corrected to the time of deposition. The tritium activity observed is indicated by the dashed line in the histogram (e). The average counting errors and their ranges (in parentheses) are: ²³⁹⁺²⁴⁰Pu, 6.8% (2.9-15); ²³⁸Pu, 24% (8-50), intervals from SNAP-9A input 8% (8-14); ²⁴¹Am, 10.4% (4.7-19). For ²¹⁰Pb, ¹³⁷Cs and ⁹⁰Sr, the counting errors were <3%.

a few hundred kilometres apart. This result suggests the site-specificity of the fluxes and the uncertainty in utilizing them as representative of a large area. Lambert et al. found a similar situation with the distribution of for in the Antarctic ice-sheet.

The lowest 238Pu/239+240Pu activity ratio occurs in Level 15,

The lowest 238 Pu/ $^{239+240}$ Pu activity ratio occurs in Level 15, 0.7% (Fig. 1c). This is concordant with the low values of the ratio (<1%) found at the Antarctic sites, J-9 and Dome C, for similar times^{1,3}.

The Arctic ratios of the integrated fluxes (²³⁹⁺²⁴⁰Pu/¹³⁷Cs, ²³⁹⁺²⁴⁰Pu/⁹⁰Sr and ¹³⁷Cs/⁹⁰Sr; Table 1) are remarkably similar for the pre- and post-moratorium intervals to those of the Antarctic site at J-9. These ratios distinctly characterize the pre- and post-moratorium testing: ²³⁹⁺²⁴⁰Pu/¹³⁷Cs, pre-moratorium 0.016 and 0.019, post-moratorium 0.012 and 0.012; ²³⁹⁺²⁴⁰Pu/⁹⁰Sr, pre-moratorium 0.023 and 0.027, post-moratorium 0.013 and 0.011; ¹³⁷Cs/⁹⁰Sr, pre-moratorium 1.6 and 1.5, post-moratorium 1.1 and 0.9. Note that an average post-moratorium activity ratio of ²³⁹⁺²⁴⁰Pu/¹³⁷Cs of 0.012 was obtained from the annual atmospheric data of Richland, Washington (1962–75), New York (1969–75) and Harwell, UK (1972–75)¹⁰. There are no data available for comparison with our pre-moratorium ratios.

The high Pu/Cs and high Pu/Sr ratios for the US dominated pre-moratorium testing period in the 1950s in the Antarctic samples¹ are confirmed here for the Dye-3 samples. Levels 14 and 15 (Fig. 1) correspond to this testing period whose fallout in the Arctic took place primarily between 1955 and 1958. The ratios have values during this period twice those of previous and subsequent years. On the other hand, the Cs/Sr ratio during this period is significantly higher than during the postmoratorium period (Table 1). The weapons debris, introduced to the stratosphere, apparently becomes mixed there in times that are short with respect to a residence time for the particles of the order of 1 yr. Clearly, the polar glaciers will record the fallout without strong distortions due to meteorology or differences in atmospheric chemistry among the different elements. Thus, as well as the low values of the ²³⁸Pu/²³⁹⁺²⁴⁰Pu ratio in the US dominated 1950s testing period we have identified another characteristic of the radioactive debris at this time.

Again, as in the Antarctic ice sheet, the recorded times of arrival of the plutonium nuclides, the caesium and strontium isotopes and the tritium follow a pattern where the transuranics are the first to accumulate, followed by the alkali and the alkaline earth nuclides and then the tritium. The percentages of the contents of nuclides in stratum 15 relative to the total in strata 13, 14, 15 and 16 are: ²³⁹⁺²⁴⁰Pu, 46; ¹³⁷Cs or ⁹⁰Sr, 31; and ³H, 17 (Fig. 1). Our original explanation indicated that the particulate transuranic nuclides have the shortest residence times in the stratosphere and the tritium, in the form of water, has the longest. ¹³⁷Cs and ⁹⁰Sr have intermediate values of the stratospheric residence times. This may relate to their hydro-

philic natures as ions and the stratospheric times involved in their abilities to form an association with waters.

The tritium concentrations in precipitation (Fig. 1e) show a pattern similar to that of ⁹⁰Sr and ¹³⁷Cs. Two distinct peaks are evident corresponding to fusion weapons testing in the mid-1950s and 1961–62. The deposition of tritium in the pre- and post-moratorium time periods is also similar to that of ⁹⁰Sr and ¹³⁷Cs. Roughly 85% of the total tritium deposition occurred in the post-moratorium period at both Dye-3 and J-9. This is due to the high production of tritium from the detonation of fusion devices¹¹.

Figure 2a displays the deposition at Dye-3 relative to that at J-9 on a year by year basis. Four major events are evident from this graph. All events indicate a sudden increase of deposition at Dye-3 relative to J-9. Note that the tritium deposition usually increases at both stations at about the same time, but the increase for Dye-3 is larger. The first peak follows the beginning of fusion bomb testing in the mid-1950s. The second event follows the large test series of 1961-62. The two smaller events seem to correlate with nuclear series of France and the People's Republic of China following the 1963 moratorium. These tests indicate that for all major series, the initial tritium deposition increased most in the northern polar region. After 1-2 yr, the extreme differences in deposition appear to approach a baseline ratio (Dye-3/J-9 deposition) of slightly greater than six. This is close to the difference in the annual precipitation at the two sites (52 cm compared with 8.9 cm) indicating very little difference in the specific activity at the two sites within about 2 yr after a test series. The small difference in specific activity could be accounted for by the higher tritium concentrations found in Northern Hemisphere surface waters¹⁷

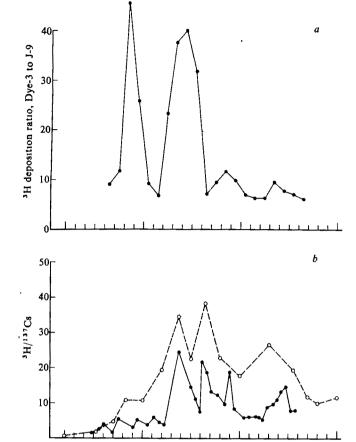
This pattern can be explained as follows. Most nuclear testing produced a large increase in the tritium burden of the northern hemisphere's troposphere and stratosphere. The tropospheric tritium quickly deposited on the Earth's surface while the stratospheric tritium began to mix into the water vapour of the northern troposphere and southern stratosphere. After the initial tropospheric input was deposited, the deposition at Dye-3 was primarily from stratospheric input, although some tritium from re-evaporation was also present. The southern polar troposphere received a much smaller initial tritium input from the nuclear tests. Most of the tritium it received came from the stratosphere. Thus, in less than 1 yr, tritium concentrations at both polar tropospheres were dependent on stratospheric input. From Fig. 2, it appears that transient stratospheric inputs level off in ~2 yr. Due to the short residence time of tritium in the atmosphere, deposition at Dye-3 was ~20 times larger than that at J-9.

Figure 2b, c shows the relative deposition of tritium versus 90 Sr and 137 Cs at both sites. As expected, very little tritium is present relative to these two isotopes in the early nuclear tests.

		239+24	°Pu	137Cs	1	90Sr		3H*		²³⁹⁺²⁴⁰ Pu	239+240Pu	137Cs
Arctic	Ref	Inventor	y (%)	Inventory	(%)	Inventory	/ (%)	Inventory	(%)	137Cs	90Sr	90Sr
Dye-3 Greenland (65°11' N, 43°50' W)	This	0.3		21 3		16 7		2.27×10^{-3}				
Post-moratorium			59		65		72		87	0 012	0 013	1.1
Pre-moratorium			41		35		28		13	0.016	0 023	1.6
South Dome, Greenland (63°31 6' N, 44°34 5' W)	2	0 7										
Post-moratorium Pre-moratorium			56 44									
Antarctic												
J-9 (82°22' S, 168°40' W)	1 '	0.04		28		2.7		1.12×10 ⁻⁴				
Post-moratorium			57		68		77		85-	0.012	0.011	09
Pre-moratorium			43		32		23		15	0 019	0 027	1.5
Dome C (74°39' S, 123°10' E)	3	0 04										
Post-moratorium			36									
Pre-moratorium		•	64									

The inventories calculated for all nuclides except ²³⁹⁺²⁴⁰Pu were corrected for decay to the time of deposition as derived from ²¹⁰Pb chronology and are in mCi km⁻².

* ³H inventory units in g km⁻².



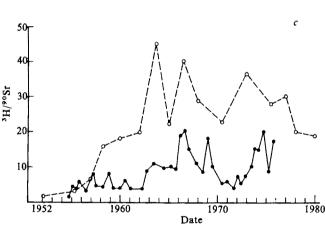


Fig. 2 a, Ratio of ³H deposition at Dye-3 to J-9 as a function of time; b, ³H/¹³⁷Cs ratio as a function of time at the Greenland and Antarctic site; c, ³H/⁹⁰Sr ratio as a function of time at the Greenland and Antarctic site. ○, Dye-3; ●, J-9.

Following the advent of fusion testing, tritium deposition increased.

These results emphasize the need to carry out similar measurements on mid-latitudinal (alpine) glaciers. We suspect that such glaciers, which exist at all latitudes of the Earth, may hold a detailed record of global artificial radioactivity fallout not found elsewhere and which should contain surprises. With the development of methods over the past decade or so to measure such nuclides at ²³⁹Pu, ²⁴⁰Pu, and ²⁴¹Pu, ²⁴²Cm, and the fission nuclides, their time and space distribution in glaciers should be most revealing about the nature of the weapons and the meteorology of transport. We recognize the difficulties in the identification of appropriate sampling sites where summer melting is negligible or non-existent such that percolation does not smear the record. Approximately 8% of each year's snowfall at Dye-3 was melted and refrozen as ice layers¹³. This suggests

that moderate melting at mid-latitude glacier sites would not seriously perturb the record of radionuclide fallout. We submit that the search for such a record will be rewarding.

This work was supported by grants from the Department of Energy, Environmental Programs, Division of Biomedical and Environmental Research and the National Science Foundation, Division of Polar Programs. H. Rufli drilled the firn cores. We thank Dr T. M. Beasley of Oregon State University for critical comments.

Received 30 November 1981, accepted 13 February 1982

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Fission product transmutation effects on high-level radioactive waste forms

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The properties of potential nuclear waste forms have been under investigation for more than a decade to ensure their safe handling, packaging, transport, storage, and disposal. Two kinds of high-level radioactive waste (HLW) have received most attention: (1) that which could be produced from reprocessed commercial reactor fuel, and (2) the slightly less radioactive waste from military activities. Both kinds would be converted to solid, nondispersible forms such as glass or crystalline ceramics for disposal. After disposal, self-radiation damage, primarily radiation-induced atomic displacements, can change the properties of these waste forms and possibly increase the chances that radioactivity could be released to the environment. β decay of the fission products causes some displacements but α decay, and the associated recoil nuclei, of the actinide elements produces several orders of magnitude more displacements. Most recent studies on radiation effects have therefore concentrated on α decay¹⁻³. A potentially more important aspect of β decay is the associated transmutation of fission products which usually results in changes of both valence and ionic size, both of which may not be readily accommodated by HLW solids. 137Cs and 90Sr are the two main sources of transmutations, but they have not been studied because of their ~30-yr half lives. The problem has now been circumvented by preparing simulated waste forms and other solids containing 134Cs, which has only a 2-yr half life. This study will not be completed for at least another year, but we report here that results obtained after 2 yr of decay are encouraging in that negligible changes have been observed in the properties of all the materials studied.

Fission products would comprise 40-90% of the HLW stream; the rest would consist of actinides and reprocessing elements. Most (~84%) of the fission products are totally non-radioactive and about half of the radioactive isotopes have half lives $\ge 10^5$ yr. Only $\sim 2\%$ have half lives ≤ 1 yr. Thus, 137 Cs and 90 Sr, which comprise $\sim 6\%$ of the fission products, are the two isotopes of major concern with regard to transmutations. Caesium decays to barium which results in a change in valence from +1 to +2 and a reduction in ionic radius of 20%. Strontium

Table 1 Density of HFIR samples and non-radioactive standards

	Material		Densit	y (g cm ⁻³)*	
No.	Type	Wt % Cs	June 1979	February 1980	June 1981
3S	Standard		3.209 ± 0.007	3.203 ± 0.004	3.214±0.007
5S	Standard	****	2.689 ± 0.009	2.690 ± 0.000	2.691 ± 0.007
1	Simulated waste glass	2.63	1.993 ± 0.018	1.990 ± 0.010	1.992 ± 0.004
2	High caesium glass with 244Cm	28.8	$2,704 \pm 0.015$	2.727 ± 0.007	2.731 ± 0.005
3	High caesium glass	31.1	2.835 ± 0.021	2.857 ± 0.018	2.911 ± 0.028
4	High silica glass	3.65	2.362 ± 0.005	2.360 ± 0.000	2.381 ± 0.015
5	High alumina glass	3.27	2.315 ± 0.004	2.310 ± 0.000	2.309 ± 0.007
7	Pollucite	42.7	2.970 ± 0.020	2.970 ± 0.000	2.994 ± 0.003
8	Supercalcine	3.16	4.207 ± 0.022	4.208 ± 0.010	4.199 ± 0.010

^{*} Errors listed represent twice the standard deviation of the mean.

decays to zirconium; thus the valence changes from +2 to +4 and the reduction in ionic radius is $\sim 29\%$. However, both ¹³⁷Cs and ⁹⁰Sr have half lives of ~ 30 yr, so it is not practical to use them to study the effects of transmutations.

Two possible ways of studying the effects of transmutations are: (1) to prepare a simulated waste using particular short-lived radioactive isotopes; and (2) to prepare a waste form with nonradioactive material and then irradiate it with neutrons to generate suitable short-lived isotopes. The first method would be prohibitively expensive; the second method is feasible with caesium but not strontium because naturally occuring isotopes of the latter have quite small neutron capture cross-sections. In fact, ¹³⁴Cs is the only suitable isotope that can be generated by neutron irradiation. All other elements have neutron capture cross-sections that are too small, the half lives of the radio-isotopes produced are too short or too long, or the decay scheme does not yield suitably large changes in valance and ionic size.

Natural caesium is 100% ¹⁵³Cs, which has a fairly large thermal neutron capture cross-section. Therefore, a reasonable fraction of the caesium can be converted through neutron irradiation to ¹³⁴Cs, which decays to barium with a 2.06-yr half life. However, because ¹³⁴Cs also has a large thermal neutron capture cross-section that yields the very long-lived ¹³⁵Cs isotope, the amount of ¹³⁴Cs produced reaches a maximum rather than continuing to increase with radiation dosage. The high flux isotope reactor (HFIR) at Oak Ridge National Laboratory, Oak Ridge, Tennessee, was the most efficient reactor available to generate ¹³⁴Cs because of its favourable neutron

energy spectrum and very high neutron flux. Calculations showed that a maximum of 20% of the caesium would be converted to 134 Cs during an irradiation period of \sim 100 days.

Seven glasses and ceramics containing caesium were irradiated in the HFIR during the first 3 months of 1979. Immediately following irradiation, all samples were annealed to remove the neutron radiation damage effects that could mask changes caused by subsequent transmutations. The glass materials were remelted at 900–1,000 °C for 30 min, and the crystalline materials were annealed at 1,150 °C for 1 h. Remelting the glasses should remove atomic displacement damage caused by the high-energy neutrons. Whether the crystalline materials were adequately annealed is not known; it was hoped that X-ray diffraction data would answer that question but those results have been unsatisfactory until quite recently.

Calculations showed, and post-irradiation measurements confirmed, that most of the activity in the irradiated samples was due to ¹³⁴Cs. Thus, any changes in properties are due to Cs→Ba transmutations with only a negligible contribution from other radioisotopes.

Post-irradiation measurements also showed that only 12% of the caesium was converted to ¹³⁴Cs rather than the 20% that had been calculated; however, this should be enough ¹³⁴Cs to observe any property changes as the caesium transmutes to barium. Post-irradiation analyses were completed immediately after the samples were annealed and are being repeated at intervals of ~1 yr. X-ray diffraction, optical and scanning electron microscopy, leach tests, and density measurements are

Material		% Of element les Boron	ached in 7 days at 90 °C	in deionized water*	Silicon	
no.		Вогон			Sincon	
110.	May 1979	April 1980	August 1981	May 1979	April 1980	August 1981
1	ND†	0.93 ± 0.17	1.61 ± 0.22	0.57 ± 0.36	1.00 ± 0.12	1.36 ± 0.31
2	23.6 ± 17.6	15.0 ± 6.6 §	22.7 ± 3.0	7.8 ± 3.4	5.7 ± 2.3 §	10.3 ± 0.4
3	27.6 ± 2.6	14.5 ± 2.8§	24.3 ± 8.9	7.0 ± 0.7	6.5 ± 1.98	7.6 ± 0.6
4	ND	0.46 ± 0.20	0.61 ± 0.07	0.13 ± 0.00	0.15 ± 0.03	0.12 ± 0.03
5	ND	0.31 ± 0.33	0.31 ± 0.15	0.43 ± 0.22	0.61 ± 0.19	0.57 ± 0.22
7	-‡	-		0.70 ± 0.30	1.15 ± 0.06	1.25 ± 0.12
8	-	-		3.1 ± 0.3	2.33 ± 0.07 §	2.97 ± 0.14
	•	Molybdenum			Caesium	
	May 1979	April 1980	August 1981	May 1979	April 1980	August 1981
	0.11 ± 0.04	ND	0.62 ± 0.26	0.52 ± 0.12	0.89 ± 0.19	1.56 ± 0.22
	16.5 ± 15.8	9.5 ± 3.6 §	14.1 ± 3.1	15.3 ± 9.5	5.99 ± 2.21 §	16.5 ± 3.6
	18.6 ± 0.9	8.8 ± 1.9 §	16.4 ± 7.5	29.9 ± 1.3	13.8 ± 2.85	28.6 ± 6.2
	· –	-		0.36 ± 0.03	0.38 ± 0.02	0.58 ± 0.07
	_	-	•••	0.53 ± 0.24	0.34 ± 0.10	0.38 ± 0.14
	_	-	•••	1.25 ± 0.51	1.68 ± 0.36	2.58 ± 0.34
	0.71 ± 0.11 §	0.09 ± 0.02	0.08 ± 0.04	2.95 ± 0.16	0.57 ± 0.26 §	1.98 ± 0.65

^{*} Errors listed represent twice the standard deviation of the mean.

[†] ND, —, element not detected.

^{‡,} Element not present.

[§] Results that are out-of-line with related results and are probably in error.

being made to determine changes as the Cs - Ba transmutations

Table 1 lists the materials irradiated, the amount of caesium in each and results of the density measurements. Density results for two non-radioactive reference materials are also included. Three to five measurements were made on each sample. Densities have increased slightly for four of the materials including pollucite. The largest increases are for the two high-caesium glasses, particularly the one without curium. Increases for the other two materials (4 and 7) are statistically significant, but just barely; additional measurements a year or so from now will be required definitely to establish a trend. No measureable change has been found for materials 1, 5, and 8.

Leach results are listed in Table 2. Three samples of each material were leach-tested in May 1979, three different samples of each were leached during April 1980 and so on. The leach tests were done at 90 °C in deionized water for 7 days. Water volume to sample surface area ratios were 30–40 cm; more importantly, this ratio was maintained at a constant value in each leach test of a given material. Following the leach tests, the leachate solutions were analysed for $^{134}\mathrm{Cs}$ activity using a high-resolution γ -ray spectrometer which was calibrated with a $^{134}\mathrm{Cs}$ standard traceable to the National Bureau of Standards.

After the leachate solutions were analysed for caesium activity, the level of radioactivity was reduced by passing the solutions through ion exchange columns to remove the caesium. The solutions were then analysed by inductively coupled plasma spectroscopy for most other cations except alkali and alkaline earths whose concentrations were altered by the ion exchange resins.

Except for the alkali and alkaline earth interferences noted above, only those elements listed in Table 2 were found in the leachate solutions in concentrations above the detection limits. Several results from the second test and one from the first test seem to be in error. If so, then the leach rates of samples 2, 3, 4, and 5 have not changed noticeably. Sample 8 also has not changed much except for a very small decrease in the leach rate of Cs. The leach rates of samples 1 and 7 have increased slightly. However, the increases are relatively unimportant from a waste management point of view as the changes are less than a factor of 10.

Interestingly, density changes were largest for samples 2 and 3 whereas their leach rates have not changed. This is probably because their leach rates are so large to start with that small changes in microstructure makes little difference.

Optical micrographs were taken of the same areas of all seven samples during each of the three post-irradiation examinations. Scanning electron micrographs were also taken during the second round of examinations and compared with electron microprobe results obtained during the first post-irradiation examination. No change in microstructure was found in any of the samples.

X-ray diffraction results have been unsatisfactory until recently when a new shielded diffractometer became operational at PNL. Only the irradiated pollucite has been examined so far, and even those results are quite preliminary. The pollucite still seems to be at least partially crystalline and the lattice parameters have not changed appreciably. These cursory results obtained about 2 yr after irradiation are thus in approximate agreement with electron diffraction results obtained on a pollucite sample about 6 months after irradiation. The new X-ray equipment will allow much more quantitative results to be obtained in the near future, and other samples, particularly the supercalcine, will also be examined.

About 6% of the total caesium had undergone transmutations at the time of the most recent examinations. In the high caesium glasses, this represents nearly twice as many $Cs \rightarrow Ba$ transmutations as would occur in most commercial waste glasses. The other three glasses have experienced 25–50% of the transmutations as would a typical waste glass. But no changes in properties were found that would be of any consequence from a waste management viewpoint. The crystalline materials were also

relatively uneffected. However, the number of transmutations in these materials has been only about 13% of those expected for an actual waste.

This work was supported by the US Department of Energy contract DE-AC06-76RLO 1830.

Regeived 19 August 1981, accepted 17 February 1982

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Synthesis of a quasi-stable kaolinite and heat capacity of interlayer water

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The mineral halloysite, in its hydrated and dehydrated forms, has attracted much attention but, in spite of much careful X-ray and electron diffraction work, and electron microscopy, NMR and IR spectroscopic studies, there remain many questions about the crystal structure of the layers, their stacking. as well as the position, motion and bonding of the water molecules (in the hydrated form) to each other and to the surrounding silicate layers. The difficulties associated with studying halloysites are principally due to the great variability observed in natural samples2 and, in the hydrated form, the existence of substantial amounts of pore water in addition to interlayer water. We have attempted to simplify the former difficulty by synthesizing a 10-Å hydrate similar to halloysite-10 Å (ref. 3) starting with a well characterized, crystalline kaolinite from Georgia. Here we report the successful synthesis of a 10-A hydrate which is stable at reduced temperatures and contains no pore water. With this material we have measured C_p for the interlayer water between 110 and 270 K.

The question of the nature of the water in fully hydrated halloysite (designated here as halloysite-10 Å) is of great interest as it can serve as a model for water absorbed on silicate surfaces. In principle, it should be relatively simple to determine the chemical and physical properties of this water because there are few, if any, cations present and there is a much smaller net electrostatic charge on the clay layers than is found among all other hydrated layer silicate minerals. Natural hydrated halloysites must be kept in contact with liquid water or they rapidly dehydrate. The existence of large amounts of pore water makes it very difficult to study the properties of the interlayer water. Our approach has been to synthesize a dihydrate (Al₂Si₂O₅(OH,F)₄·2H₂O) starting with well characterized kaolinites. This guarantees a reproducible material of known morphology, crystallinity and purity. The new synthesis produces a more stable product which is virtually free of pore water and thus represents a considerably simpler material from the point of view of spectroscopic and thermal analysis measurements.

We have previously described³ a means of producing a 10-Å hydrated kaolinite which is indistinguishable from natural halloysite-10 Å samples from Utah. As with the natural mineral,

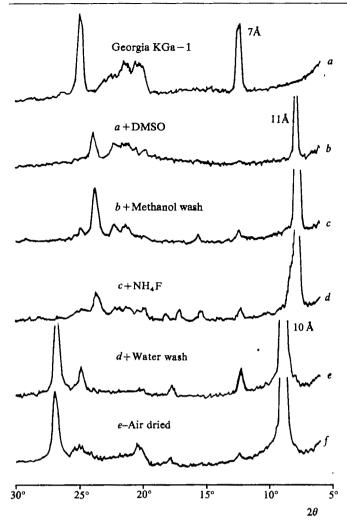


Fig. 1 X-ray diffractograms showing the original kaolinite (a), the intermediate stages of the synthesis (b-d) and the 10-Å product when wet (e) and dried (f). The diffractograms were made with CuK radiation.

this synthetic hydrate must be kept in contact with water to prevent spontaneous dehydration. In spectroscopic studies, such as IR and NMR, it is difficult, if not impossible, to distinguish between the pore water and the intercalated water.

The new synthesis is a variant of the method reported earlier. Figure 1 shows the sequence of steps in terms of the X-ray diffractograms of the starting material, the intermediate stages and the final dried product. The starting clay is the Georgia well crystallized kaolinite identified as KGa-1 (ref. 4). The clay is expanded with dimethyl sulphoxide (DMSO) containing 8% water (step b, Fig. 1) after which the intercalate is washed with methanol (step c, Fig. 1). The key step, as before, is the treatment of the intercalate (step d, Fig. 1) with ammonium fluoride (400 mg per g of kaolinite) for a period of several hours at 60 °C. This is followed by repeated water washings to remove the ammonium fluoride and intercalated DMSO. The wet clay is allowed to dry in ambient conditions. Complete loss of pore water may take several days. As reported earlier³, there is probably fluorine replacement of some surface hydroxyls. We are currently determining the extent of this substitution and its influence on hydrate formation.

Thermogravimetric analysis studies indicate a weight loss of 10% between 300 and 500 K. The theoretical weight loss for a dihydrate is 12.2%. Because X-ray diffraction indicates a yield of $\sim 90\%$, the observed weight loss is in good agreement with the expected value. The remaining 10% is clay which has collapsed back to kaolinite. Moreover, thermogravimetric

analysis does not indicate any substantial amount of pore water. The synthetic hydrate is a hard, dry, massive material which will remain fully hydrated for periods of several months if kept in a sealed container at temperatures near 0 °C. It is not stable at room temperature and in fact loses water rapidly beginning at 280 K. Because of its instability at room temperature, we refer to the synthetic material as a quasi-stable kaolinite dihydrate.

To characterize the interlayer water further, we have studied the IR spectrum and taken measurements of the heat capacity (C_p) . A more detailed discussion of these and other experiments (NMR and electron paramagnetic resonance) will be reported elsewhere.

The IR spectra (Fig. 2) were recorded on a Pye-Unicam 3-300 spectrometer with the clay dispersed in a fluorolube mull. The major features of the stretching bands of water and hydroxyls as well as the deformation bands of water are shown along with the corresponding spectra for the starting clay and for a natural halloysite-10 Å. The IR spectrum of the quasistable kaolinite dihydrate is clearly very similar to that of the natural 10-Å material. The greater crystallinity of the Georgia kaolinite starting material and the absence of pore water allow us to discern details in the spectrum of the synthetic hydrate which are difficult to see in the natural material. The broad band centred around 3,400 cm⁻¹ is due to the continuous water layer⁵. In addition, one sees bands at 3,536 and 3,586 cm⁻¹ which have been reported before⁵ but are very clearly resolved in our material. As will be discussed in detail elsewhere, these bands correspond to water molecules attached to di-trigonal sites on the tetrahedral surface of the interlayer space; we refer to these molecules as 'di-trigonal hole' water. The identification of the nature of the di-trigonal hole water has been facilitated by the existence of a kaolinite monohydrate (d(001) = 8.4 Å)which is the dehydration product of the quasi-stable kaolinite-10 Å. Further evidence for the two types of water (di-trigonal hole and continuous monolayer) can be seen in the partially resolved doublet corresponding to the bending mode.

As noted by Cruz et al.⁶, there are only small shifts in the structural OH stretching frequencies between kaolinite and halloysite-10 Å. The same observation is true for the quasi-stable kaolinite dihydrate and its parent kaolinite (Fig. 2). This suggests that the surface hydroxyls are bonded to water molecules in much the same manner that they bond to the oxygens of the adjacent kaolinite layer.

Cruz et al.⁶ reported heat capacity (C_p) measurements of the water in a natural endellite (halloysite-10 Å). Our C_p measure-

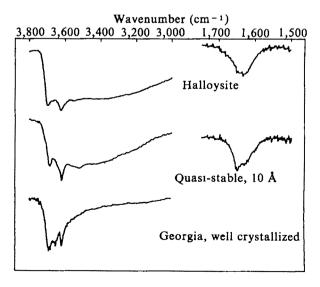
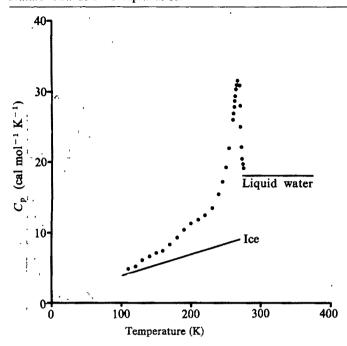


Fig. 2 IR spectra of the Georgia kaolinite (CMS-KGa), the synthetic 10 Å hydrate and a naturally occurring hydrated halloy-site (Utah). The OH stretching and the water bending regions are shown.



A plot of heat capacity versus temperature for the interlayer water of the quasi-stable kaolinite 10 Å.

ments were made on four samples (two between 110 and 190 K and two between 190 and 275 K) of the quasi-stable kaolinite-10 Å using a Perkin-Elmer DSC-2 scanning calorimeter equipped for low temperature operation. The experiment technique was that used by Cruz et al.⁶. The C_p values are plotted in Fig. 3. Internal agreement between different samples is of the order of 2-5% except for the very high C_p values in the vicinity of 265 K where the discrepancy is nearer 10%.

At the lowest temperatures, C_p values for the 10-A water lie very close to values found for ice⁷. Beginning at $\sim 160 \text{ K}$ the C_p values move away from the ice line with a break in slope in the vicinity of 240 K. Above 240 K, C_p rises abruptly to a maximum at 260 K. This peak is reminiscent of the observations of Mraw and Naas-O'Rourke8 on 'freezable' pore water in coal. Cruz et al. did not report this peak in endellite because the upper temperature of their measurements was 255 K. By contrast, C_p for di-trigonal hole water alone, as measured in the kaolinite (8.4 Å) monohydrate, lies very close to the ice line throughout the temperature range studied but shows no discontinuity at 273 K. IR and NMR- $(T_1 \text{ spin-lattice relaxation time})$ measurements as a function of temperature suggest that the heat capacity peak of the kaolinite-10 Å water is associated with an 'order-disorder' type transition in which hole water is transferred to the continuous water monolayers as the temperature is raised. Note that one can readily distinguish two populations of interlayer water in the quasi-stable kaolinite 10 Å.

We thank Dr R. Prost, Dr M. Cruz-Fripiat and Professor J. J. Fripiat for their comments and suggestions concerning our investigation of water in kaolin minerals. Part of the research was supported by a grant from NSF/Sciences to R.F.G.

Received 23 November 1981, accepted 5 February 1982

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Volatiles in phyllosilicate minerals

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Volatiles other than water are known to occur in hydrous ringstructure silicates like beryl and cordierite1.2. However, there has been limited study of volatiles in phyllosilicates. Ammonia has been reported in micas3.4; Banks⁸ measured sulphur in biotite, sericite and chlorite by electron microprobe; and Norman⁶ reported evolution of H₂S and CO₂ when chlorite, sericite and kaolinite were heated to 500 °C. We have studied the thermal evolution of volatiles from phyllosilicates by mass spectrometry. The gases observed were, in approximate order of their abundance, CO₂, H₂S, H₂, SO₂, NH₃, CO, CH₄, N₂, Ar and He. These gases represent 0.35-9 wt % of the volatiles observed; the remainder is water. Step-heating studies indicate H₂S and CO₂ are not generated by the thermal breakdown of sulphide and carbonate minerals. Rather, they are bound within the phyllosilicate structures in a complex way. We show here that gases in chlorite, muscovite and biotite are related to their mode of origin. Phyllosilicates from hydrothermal ore deposits evolve several times more volatiles, principally CO2 and H2S, than phyllosilicates not from ore deposits.

Mineral separates were hand-picked, ground to -70+160mesh, rinsed in distilled water, separated by heavy liquids, treated with warm 10% HCl for 15 min, multiply rinsed and oven dried. Samples were examined for impurities by scanning electron microscope (SEM) with energy dispersive detector, and loaded into a 5×40 mm quartz tube which was placed in an externally heated quartz furnace connected to a vacuum system. The sample was baked at 125 °C while being evacuated to 10⁻⁶ torr. Decrepitation was by heating to 700 °C for 1 h, then 1,100 °C for 1 h. Gases were determined by pressure measurement and quadrupole mass spectrometer; water was measured by weighing. Step-heating samples were heated for 30 min at 100 °C intervals and the evolved volatiles were analysed after each step.

Volatiles evolved on heating belong to five groups, water (including H₂), carbon gases, sulphur gases, nitrogen gases and rare gases. The C-S-N gases vary from 0.01 to 0.33 wt % of the phyllosilicates and analysed and 0.27-9.0 wt % of the volatiles released (Table 1). Within each group of gases, the ratio of oxidized to reduced species depends on the heating procedures. Step-heated samples yield more reduced gases than those heated in two steps.

The gases observed are related to the mode of origin of the mineral. Phyllosilicates from ore deposits evolve several times more gases than those not from ore deposits. In particular, chlorite associated with ore deposits evolves up to 10 times the CO₂ as other chlorite, and micas from ore deposits evolve up to 50 times more H₂S than micas from other environments.

Of particular concern was that the carbon and sulphur gases measured resulted from thermal breakdown of sulphide and carbonate mineral inclusions. Contamination of the mineral separates by sulphide and carbonate minerals is not considered to be serious because contaminate minerals, particularly sulphides, were readily detected by SEM examination and several chlorite samples from rock with a high percentage of sulphide minerals vielded little sulphur gases.

To investigate mineral inclusions and contaminate minerals as sources of carbon and sulphur gases, step-heating studies were done. Evolution of H₂S and CO₂ from phyllosilicate minerals is complex, similar to water (Fig. 1). Hydrogen sulphide is the principal sulphur gas with SO₂ evolution occurring primarily at $T \ge 1,000$ °C.

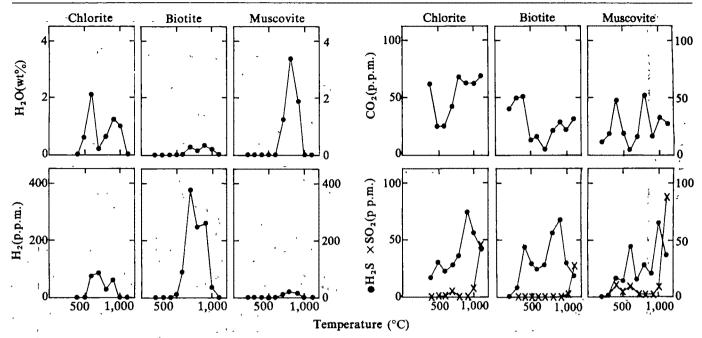


Fig. 1 Evolution of the principal volatiles when three different phyllosilicates were step heated. Chloride is sample CHL2A from a chlorite schist. Muscovite is sample MUS2B from the Bjertnes pegmatite, Norway. Biotite is sample BIO2A from a pegmatite near Mora, New Mexico, USA.

In contrast, when ground sulphides were heated with a low-sulphur chlorite the thermal release curves are simple, SO₂ is the predominant sulphur gas, and considerable SO₂ is generated at temperatures <1,000 °C (Fig. 2). When other mixtures of sulphides, which included FeS, FeS₂, CuFeS₂ and PbS, were heated with chlorite similar results were obtained. Carbon dioxide generated by the thermal breakdown of carbonates similarly has a single thermal release curve which peaks at 700 °C (Fig. 2).

We conclude that the sulphur and carbon gases released on heating phyllosilicates are not the result of mineral inclusions

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or contaminate minerals because: (1) The thermal release of sulphur gases and CO_2 from phyllosilicates does not resemble the generation of these gases in our experiments. (2) Thermal release curves for H_2S and CO_2 are complex, with several peaks, and are similar to water which is structurally bound in phyllosilicates. (3) Contaminate sulphides evolve more SO_2 when heated with phyllosilicates and the SO_2 is generated at $T < 1,000^\circ$; whereas phyllosilicates evolve mostly H_2S and SO_2 is formed at $T > 1,000^\circ$. (4) Carbon dioxide thermal-release curves have a minimum at the temperatures at which carbonates breakdown; 700-800 °C. (5) Gases bound to surfaces would be

. Table 1 Volatiles released when phyllosilicates were heated to 1,100 °C

			,	,									(exclud	ling H ₂)
Sample no. and location	H ₂ O (wt %)	, H ₂	CO ₂	co ·	,CH4	C ₂ H ₆ (p p.m)	H _z S	SO ₂	N ₂	NH ₃	Ar (p.	He p.b.)	wt %	gas/ H ₂ Q ×100
Chlorite	1 3									-	,			
PM-2A Pecos, NM USA (o)	7.50	<u> </u>	2,200	240	34		15	67	63	`		NA '	0.26	3.5
14-TBG-74 Tribag, Ontario,					•	11 1					-			+
Canada (o)	10.1	_	1,800	140	15		, 31	2.7			*****	NA	, 0.20	2.0
CHL4A Buchans, NF Canada (o)	11.1	20	1,500	150	230	,	, 52	25	36		~~~		0.20	1.8
CHL3A chlorite schist			٠ , ـ											
no. NM USA	10.7	42	380	17			490	240		28		NA	0 12	1.1
CHL3A (s)	10.1	260	420	, 14	. 42		310	76		51		NA.	0.09	0.87
CHL2F meta-komatiite,														
NM USA	11.0	713	220	3.9	1 59	40 ,	79	7.1	9.1	,		-	0.03	0 27
Muscovite	*.	77			•									r
C5B Creede, CO USA (o)	4.84	100	870	1,100	83	 ,	1,100	130				NA	0.33	6.8
MUS2B Bjertnes pegmatite,							•	,			•			
Norway, .	6.90	5.2	530-	190	110		130	170	******		•	NA	0.11	1.6
MUS2B (s)	6.70	82	250	130	93	380 1	240	130	` <u>`</u>		٠	NA	0.12	1.8
MUS4A Sericite schist,							-			, ,				21
no. NM, USA	0.76	8.8	86	· 55	7.4	25	2.2	0.6	75			4	0.01	1.3
MUS3A Pegmatite, NH USA	7.92	. 20	110	·190	28	30		6.0		-	400	20	0.04	0.51
Biotite														
B101B Questa, NM USA (o)	2.70	110	170	490	26		1,000	500	31	226		NA	0.24	9.0
SR1A Santa Rita, NM USA (o)	2.90	340	180	14	2.0		880	65		10	******	NA	0.12	4.1
SR1B Santa Rita, NM USA (o)	1.60	47	730	220	140	82	160	44	27		1.2	0.4	0.14	8.8
CSG1A Cold Spring granite,					•									0.0
MN, USA	`2.80	320	210	: 45	44	24 -	- 170	58	14	5.9	72	-	0 05	1.8
MD1A Mayo-Darle Sn-granite														
Cameroon	. 7.96	55	110		45	27	17	5.2	23		32		0.05	0.63
B102A Pegmatite,	,	-	•											
no. NM USA	1.21	1,100	- 280	140	9.0		310	26		. 14	***	, NA	0.08	6.6

Analyses of step-heated samples (s) and samples from ore deposits (o). Data are presented as the weight ratio of volatile to mineral. NA, not analysed

released at T < 500 °C and hence cannot account for most of the gases observed.

It is unlikely that volatiles other than rare gases occur in the phyllosilicates in the form they are measured. For example, water occurs as the hydroxyl ion. Neither the species which yield the gases observed nor their structural location can be deduced from the data presented or from calculated activation energies. There is evidence that nitrogen occurs as the NH4 ioff bound between silicate layers^{4,7}. Carbon dioxide production has been demonstrated by heating silicate minerals containing elemental carbon⁸; however, there is no reason to exclude the possibility that carbonate or bicarbonate ions might occur in phyllosilicates in a manner similar to ammonia. Our data suggest that water and hydrogen are released from chlorite and biotite at two temperatures by the breakdown of Fe-OH and Al-OH bonds; whereas these volatiles are evolved from muscovite primarily at one temperature by the breakdown of Al-OH bonds. Evolution of H₂S and CO₂ is not analogous to water in that they have multiple-peaked, thermal-release curves for muscovite. This suggests that H₂S and CO₂ are not structurally bound in the same way as water, although the temperature of gas release suggests bond energies for H₂S and CO₂ the same as the hydroxyl ion.

The association of high amounts of volatiles in phyllosilicates with ore deposits suggests that volatiles in phyllosilicates have

Received 18 December 1981, accepted 21 January 1982

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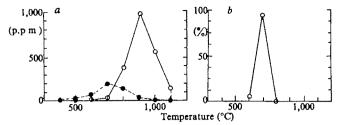


Fig. 2 a, An example of step-heating chlorite to which finely ground sulphides were added. O, SO2; O, H2S. These data are for chlorite sample CHL2F to which was added ~0.35 wt % of a mixture of pyrite and chalcopyrite. b, Evolution of CO₂ (O) from finely ground calcite when it was step-heated in an analogous manner to the phyllosilicates

a potential use as an exploration tool for ore mineralization. The ubiquitous presence of sulphur in phyllosilicates may be an unrecognized source of reduced sulphur. This reduced sulphur could be an important source of sulphur in the genesis of some ore deposits. Also, phyllosilicates, either during formation or through ion exchange processes, could be important in the inorganic reduction of sulphate.

This work was supported by Office of Surface Mining, US Department of the Interior grant no. G5104024.

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Seismicity and rheology of subducted slabs

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Since the incorporation of the inclined zones of intermediate and deep seismic activity into the theory of plate tectonics1,2 it has been generally accepted that earthquakes with focal depths ≥100 km occur in subducted slabs of (predominantly oceanic) lithosphere. Yet our understanding of the processes which generate intermediate and, in particular, deep earthquakes is still incomplete. This is largely due to our limited knowledge of the rheological behaviour of subducted oceanic lithosphere at depths between ~100 and 700 km. However, recently considerable insight has been gained into the rheology of oceanic lithosphere in near-surface conditions and I investigate here whether the seismic activity at depths >100 km can be understood on the basis of this information. I first present results of temperature calculations performed for all subduction zones for which adequate data on seismicity, relative plate motion and age of the descending oceanic lithosphere exist. Subsequently, I adapt the temperature conditions governing the rheology near the surface by including depth-dependence. This leads to a depth-dependent critical temperature $T_{cr}(z)$ above which the subducted lithosphere cannot sustain the stresses necessary to generate seismic events. Seismicity data and calculated temperature versus depth relationships are in good agreement with the rheological model predictions. This implies that the absence of seismic activity at depths ≥700 km should not be interpreted as direct evidence for the hypothesis that the 650-km discontinuity in the mantle acts as a barrier to vertical motion of subducted slabs.

McKenzie³ and Griggs⁴ investigated whether the maximum depth of earthquakes in subducted slabs is determined by a critical temperature. Although their results lent qualitative sup-

port to such a relationship their studies were based on limited data and hampered by great uncertainties concerning important geophysical parameters. Vlaar and Wortel⁵ have shown, from observational data, that the maximum depth of earthquakes in subduction zones increases with increasing age of the downgoing oceanic lithosphere. Meanwhile, results of modelling the thermal structure of oceanic lithosphere^{6,7}, description of global relative plate motion⁸ and the widespread identification of magnetic anomalies in the oceans have provided the basis for more extensive and reliable calculations of the thermal structure of subducted slabs. The calculations reported here are based on McKenzie's approach³ in which a slab is assumed to sink in a mantle with an adiabatic temperature gradient. The adiabatic temperature distribution in the (upper) mantle below the lithosphere corresponds with a constant potential temperature $T_{\rm m}^{(p)}$ (see ref. 3), for which we take $T_{\rm m}^{(p)} = 1,200^{\circ}$ C. Crough's model for the thermal evolution of oceanic lithosphere, with a temperature of 1,200 °C at the base of the lithosphere and fitted to bathymetry and heatflow data⁶, was used to calculate the age-dependent initial temperature distribution and thickness of the downgoing slab. This model implies an increase in lithospheric thickness from near-zero at a spreading centre to 101 km for 100 Myr old lithosphere. Apart from conduction and adiabatic compression the latent heat associated with the olivine-spinel phase change¹⁰, resulting in an increase in temperature of 135 °C, was taken into account.

Several aspects of McKenzie's analytical model were critically examined by using a finite difference version of the heat conduction equation. Among these was the use of a constant thermal conductivity and the assumption of stationary input of the subduction process (constant lithospheric age at the trench). Model calculations with temperature-dependent thermal parameters as given by Schatz and Simmons¹¹ seemed to yield solutions which very closely agree with those for a constant diffusivity $\kappa = 0.9 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$. The maximum difference in minimum temperature (in °C) was ~2%, the calculations with temperature-dependent parameters giving the lower values. The constant value $\kappa = 0.9 \times 10^{-6} \,\mathrm{m}^2 \,\mathrm{s}^{-1}$ was used in the final calculations. Several subduction zones exhibit temporal variations in the age of the downbending lithosphere at the trench,

Table 1 Subduction zones and parameters

	Zone	Age (Myr)	$v_{\rm c}^{(n)*}$ (cm yr ⁻¹)	Length (km)	Dip (deg)	Depth (km)	Ref.†	
1	Kuriles	100	7.9	880	50	660	26	
2	Northern Honshu	$125 \rightarrow 100^{\ddagger}$	9.7	1300	30 ,	,605	2	
3	Izu/Bonin	$145 \to 125$	5.6	- 830	50-70	580	33	
4	Ryukyus	60	5.6 ⁴	450	45	275	33	
5	Sumatra	70 → 60	5.8	400	35	200	_ 34	
6	Java	140 → 115	6.9	860	. 75	650	35	
7	Tonga (20 °S)	100-140	8.9	·¹′ 960	48	680	36	
-8	Tonga (24 °S)	100-140	8.1	865	53	640	36	
9	Kermadec (28 °S)	100-140	. 7.0	970	50	660	36	
10	New Zealand	100-140	4.7	585	60	370	37≝	
11	New Zealand	100-140	4.4	490	55	290	37 ¹	
12	Chile (36–42 °S)	35	10.6	495	20	180	38	
13	Chile (27–33 °S)	48 → 60	10.4	760	10	180	38	
14	Chile (18–26 °S)	53 → 70	10.1	680	30	320	38	
15	North-central Peru	45 → 65	9.1	750	10	185	38	
16	Central America (87 °W)	40	8.4	275	65	- 200	39	
17	Central America (90 °W)	40	7.6	355	60	250	40	
18		40 → 35	6.0	240 [¶]	≲30	172	41,42	
19	Aleutians (180 °W)	63 → 50	4.8	360	60	240	43	

^{*} Component of convergence rate normal to plate contact.

for example, Sumatra, the Aleutians and South America (see ref. 12). Investigating the effect of these variations showed that the temperature distribution at a certain downdip distance in the subducted slab depends primarily on two parameters of the part of the lithosphere involved—the temperature distribution, or age, at the time of initial downbending at the trench and the time elapsed since it started to descend. Whether adjacent parts of the slab were slightly older or younger at their times of onset of subduction is negligible.

All subduction zones for which adequate data on slab geometry (length, dip and maximum depth of seismic zone), relevant plate kinematics and age of the downgoing slab were available are listed in Table 1. The main reason for rejecting subduction zones was lack of knowledge of real convergence rates, usually because of the unknown contribution of back-arc spreading. The relative plate motions given by Minster et al. and Jordan⁸ were used instead of the more recent results by Minster and Jordan¹³ because the former are more representative of the timespan which is involved in the subduction process. Age data were primarily taken from ref. 9. Seismicity data were taken from regional studies which report high-quality hypocentre locations (references are given in Table 1). See ref. 14 for a more extensive account of the data selection and the thermal modelling procedure.

In the present context the most relevant feature of the temperature distribution in a subducted slab is the minimum temperature at the depth of the deepest earthquake. For the subduction zones listed in Table 1 these temperatures are plotted in Fig. 1 as a function of depth. Owing to the nature of the thermal evolution of oceanic lithosphere the temperature distribution in a young descending slab is more sensitive to absolute errors in lithospheric age than the distribution in an old slab. For subducted slabs 35, 65 and 120 Myr old, an error of $\pm 10\,\mathrm{Myr}$ in age results in errors in minimum temperature at the deepest earthquake foci of ±55 °C, ±25 °C and ±15 °C respectively (apart from the latent heat contribution of the olivine-spinel phase change). The uncertainty in the age of the downgoing slab in zones 7-11 (see Table 1) results only in an uncertainty of ±30 °C with respect to the values plotted in Fig. 1, which were calculated for an age of 120 Myr. Reliable calculations can only be made for continuous slabs. Therefore, the deep earthquakes beneath New Zealand (zone 11) and South America (zones 13, 14, 15), which probably occurred in

detached pieces of lithosphere, were not taken into account. In general, Fig. 1 displays a distinct increase in minimum temperature with depth. Molnar et al. 15 have produced results which are qualitatively fairly similar to those in Fig. 1, but they did not concentrate on testing the conditions that govern the extent of a seismic zone.

The basis of the present discussion is that the rheology of the seismically active part of a slab must allow for the accumulation of stresses which are relaxed in the process of an

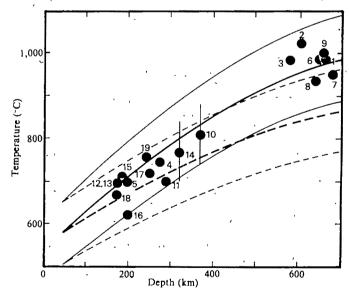


Fig. 1 Minimum temperatures at the depths of the deepest earthquakes in subduction zones: numbers refer to the zones listed in Table 1. The vertical bars for zones 10 and 14 reflect the uncertainty as to whether the olivine-spinel phase change has taken place. The points for zones 12 and 13 coincide. The thick solid curve represents $T_{\rm cr}$ (critical temperature above which no earthquakes are generated) as a function of depth, based on Stacey's mantle solidus. The thinner upper and lower solid curves indicate the range for $T_{\rm cr}$ resulting from uncertainties in the temperature at the depth of the 'calibration event' near the Tonga trench (see text). The thick dashed curve represents an alternative $T_{\rm cr}$ curve based on equation (10); the two other dashed curves indicate the uncertainty range for this $T_{\rm cr}$ curve.

[†] References from which seismicity data (downdip length, dip and maximum depth) were taken. Length is measured from the trench to the depth of the deepest hypocentres. The dip angle is measured in the intermediate depth range.

 $[\]ddagger n_1 \rightarrow n_2$ means that the present age at the trench is n_1 Myr and the deepest seismically active part of the slab was n_2 Myr old at the time it started to descend. n_2 was used in the thermal calculations.

From ref. 32.

Zones 10 and 11 correspond with Hamilton and Gale's 37 sections B and C respectively

Including a correction of 200 km for the excessively long accretionary prism.

earthquake. The problem is approached by relating the seismicity in the descending slab to relevant near-surface conditions and phenomena of which more direct observations are available. Two types of observation are considered: (1) observations of the geometry of the bending lithosphere near the trenches (in combination with those on seamount loading)^{16,17}, and (2) observations of the seismic activity of the oceanic lithosphere just before the latter starts to descend into the trench^{18,19}. Both data sets bear on the rheology of the lithosphere at the surface and, hence, on the input of the subduction process (section A in Fig. 2).

The rheological model used by Caldwell and Turcotte¹⁶ for the bending and seamount loading is simpler than that used by Bodine *et al.*¹⁷. In view of the many uncertainties attached to the slab's rheology at greater depths it seems more appropriate to base the analysis on Caldwell and Turcotte's¹⁶ model. These authors assumed that below a certain temperature T_{\bullet} elastic stresses do not relax on geological time scales whereas at temperatures above T_{\bullet} stresses are relaxed by creep processes. In this spirit Caldwell and Turcotte¹⁶ studied the rheology of oceanic lithosphere, using power-law creep behaviour for olivine appropriate for differential stresses below 2 kbar (ref. 20)

$$\frac{\mathrm{d}s_{\mathrm{p}}}{\mathrm{d}t} = C\sigma^{3} \exp(-A/RT) \tag{1}$$

 $\mathrm{d} s_\mathrm{p}/\mathrm{d} t$ is the creep rate, σ the differential stress, T absolute temperature, R the universal gas constant, A an activation volume and C a constant. By using a Maxwell-type of viscoelastic model they derived an expression for T_e :

$$T_{\mathbf{o}} = A/\{R \ln \left(\frac{2}{3}EC\tau_{\mathbf{R}}\sigma_{\mathbf{0}}^{2}\right)\} \tag{2}$$

where E is Young's modulus and τ_R is the time for the stress σ_0 , applied at t=0, to relax to one-half its original value. Using Goetze's²⁰ values A=122 kcal mol⁻¹ and C=70 bar⁻³ s⁻¹, and $E=6.5\times 10^{10}$ N m⁻², for $0.1<\sigma_0<2.0$ kbar and $0.1<\tau_R<10.0$ Myr T_e varies from 660 to 840 °C. These temperatures are in good agreement with those derived, from studies of lithospheric bending¹⁶, for the base of the elastic upper part of the lithosphere and also for the depth corresponding with Bodine et al.'s¹⁷ mechanical thickness H of the lithosphere (defined as the depth below which the yield strength of the lithosphere is <500 bar).

The usual way²¹ to include depth- or pressure-dependence in equation (1) is to put

$$\frac{\mathrm{d}\varepsilon_{\mathrm{p}}}{\mathrm{d}t} = C\sigma^{3} \exp\left(-gT_{\mathrm{m}}/T\right) \tag{3}$$

where g is a constant and T_m the melting temperature (in K) at the appropriate depth or pressure. The analogue of equation (2) is then

$$T_0 = gT_{\rm m}/\ln\left(\frac{2}{3}EC\tau_{\rm R}\sigma_0^2\right) \tag{4}$$

For $0.1 < \tau_R < 10.0$ Myr and $0.1 < \sigma_0 < 2.0$ kbar we find

$$55.2 < \ln \left(\frac{2}{3}EC\tau_{\rm R}\sigma_0^2\right) < 65.8 \tag{5}$$

As a good approximation, we may write instead of equation (4):

$$T_o \simeq \alpha T_m$$
 with $\alpha = g/60$ (6)

Briefly, studies of the bending of oceanic lithosphere have shown that the rheology of the lithosphere is strongly temperature dependent. The temperature-dependence is controlled by the ratio $T/T_{\rm m}$.

The second data set comprises precise depth determinations of earthquakes associated with the bending of the lithosphere near the trenches given by Chapple and Forsyth¹⁸ (see also ref. 19). From this data set we can derive an estimate of the maximum temperature at which deformation of the lithosphere (in near-surface conditions) produces earthquakes. This maximum temperature is found for the deepest event of the data set, which occurred east of the Tonga trench at a depth

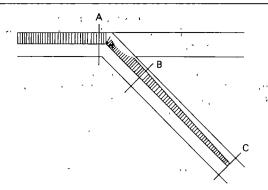


Fig. 2 Representation of a subducted slab. Hatching indicates the parts of the downgoing slab which are potentially seismically active. Earthquakes at or near the contact between the descending slab and the upper plate are not considered, in view of their different character. The lithosphere at section A is representative of the input of the subduction process. Section B and C are at the depth of the double seismic zones (about 120-200 km) and at the downdip end of the seismic zone respectively

of 45±5 km. Its focal mechanism solution (horizontal compression) indicates that it took place below the neutral surface of the bending plate. The age of the lithosphere at the Tonga trench, which determines the temperature distribution, is estimated to be in the range 100-140 Myr. According to Crough's 7 thermal model the temperature at the hypocentral depth of this event is 580±70 °C, the error being the r.m.s. error due to the uncertainties in lithospheric age and focal depth. This temperature is taken to be the (empirical) maximum temperature, designated as T_{cr}, at which the deformation of oceanic lithosphere is accompanied by seismic activity. For the analysis of intermediate and deep seismicity we are interested in the depthdependence of $T_{\rm cr}$. Because the compressive 'bending' earthquake occurred in the depth range of the lithosphere of which the rheology is controlled by temperature and on the basis of the results obtained for T_c (see equations (4)-(6)), we assume

$$T_{\rm cr}(z) = \beta T_{\rm m}(z) \tag{7}$$

where $T_{\rm m}$ is the melting temperature and β is a constant. For a chosen $T_{\rm m}(z)$ curve β can be determined from the intercept-value, that is $T_{\rm cr} = 580 \pm 70$ °C at the depth of the Tonga event ($\simeq 45$ km). For $T_{\rm m}$ we first take Stacey's²² composite mantle solidus $T_{\rm s}$. For β we find $\beta = 0.54 \pm 0.04 = \beta_{\rm s}$. $T_{\rm cr}(z)$ and the temperature range resulting from the uncertainty in β are plotted in Fig. 1. As pointed out by Anderson and Minster²³, the composite mantle solidus is representative of the low melting point phases in the polyphase mantle system. The effect of different choices for $T_{\rm m}$ can be illustrated by using, as an extreme alternative, the liquidus for which we take 2,163K at the surface. This yields a lower value of β : $\beta = 0.38 \pm 0.03 = \beta_{\rm i}$. According to Kennedy and Higgins²⁴ the liquidus temperature of the mantle increases more rapidly with depth than the solidus temperature, although in the upper mantle down to 700 km this effect may not be very pronounced. Thus

$$\frac{\mathrm{d}T_{\mathrm{l}}}{\mathrm{d}z} \gtrsim \frac{\mathrm{d}T_{\mathrm{s}}}{\mathrm{d}z} \tag{8}$$

But, because $\beta_1/\beta_1 \approx 0.7$, we have

$$\beta_1 \frac{\mathrm{d}T_1}{\mathrm{d}z} < \beta_1 \frac{\mathrm{d}T_2}{\mathrm{d}z} \tag{9}$$

The upper bound of the temperature range for $T_{cr}(z)$, based on Stacey's mantle solidus, is taken as an upper bound for all T_{cr} curves to be considered. A lower bound can be constructed by taking

$$\frac{\mathrm{d}T_{\mathrm{cr}}}{\mathrm{d}z} = \beta_1 \frac{\mathrm{d}T_{\mathrm{s}}}{\mathrm{d}z} \tag{10}$$

The T_{cr} curve based on equation (10) is also plotted in Fig. 1 (thick dashed curve). The two other dashed curves (upper and lower) indicate the range of T_{cr} resulting from the uncertainty

In the downbending lithosphere near the trench the minimum temperature is clearly below T_{cr}. From numerical model calculations I infer that, for relevant parameter values, the minimum temperature T_{\min} in a subducted slab satisfies

$$\frac{\mathrm{d}T_{\mathrm{mm}}(z)}{\mathrm{d}z} > \frac{\mathrm{d}T_{\mathrm{cr}}(z)}{\mathrm{d}z} \tag{11}$$

for both T_{cr} curves shown in Fig. 1. Because $T_{mm}(z)$ depends on the initial temperature distribution (or age) of the downgoing slab, the direction and rate of plate convergence and the dip of the slab, the depth at which $T_{\text{min}}(z) = T_{\text{cr}}(z)$ varies from one zone to another. Whether the downdip extent of a seismic zone is determined by a limiting temperature T_{cr} can now be checked at the level of section C in Fig. 2: the minimum temperatures at the depths of the deepest foci in continuous subducted slabs, plotted as a function of depth, should delineate the T_{cr} curve. Figure 1 shows that all calculated minimum temperatures fall between the upper and lower bounds derived for T_{cr} . Even with the more restricted range belonging to Stacey's solidus curve the temperatures are in very good agreement. Note that takinginto account the effect of stress level variations [see equation (5)] or errors in the melting point curve would broaden the model range for T_{cr} .

An additional check of the model (seismic activity confined to regions with $T < T_{cr}$) can be made at the level of section B in Fig. 2 (120-200 km depth), where detailed hypocentral location procedures have revealed a double seismic zone²⁵⁻²⁷. The distance between the two parallel zones varies from ~25 km for the Aleutians²⁵ to 30-40 km for the Kuriles²⁶ and Honshu²⁷ The temperature distributions I have calculated for the subducted slabs in these regions show cold zones ($T < T_{cr}$) with thicknesses exceeding the above cited values by 5-10 km.

Therefore, I conclude that the gross features of subduction zone seismicity at depths between ~100 and 700 km are adequately explained by a model in which seismic activity is confined to those parts of the subducted slabs which have -temperatures below a depth-dependent critical value T_{cr}

For many years, deep seismic activity has influenced debates about the depth extent of mantle convection²⁸⁻³¹. From the results reported here I conclude that the absence of seismic activity at depths >700 km as such should not be considered as direct evidence for the hypothesis that subducted slabs cannot penetrate the 650-km mantle discontinuity.

On the basis of our knowledge of the rheology of oceanic lithosphere and melting temperatures in the mantle, the maximum depth of earthquakes can be understood in terms of thermal assimilation or rheological de-activation.

Arguments involving the depth variations of earthquake magnitudes or energy release have not been used here in order to avoid confussion; the slab's rheology as considered here must provide basic conditions for generation of earthquakes, whereas magnitudes and energy release also depend on the forces acting on the slab and the physical mechanism of an earthquake.

I thank Professor N. J. Vlaar and Dr S. Cloetingh for discussions and comments and Dr D. W. Forsyth for a preprint.

Received 24 November 1981, accepted 17 February 1982

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Indonesian Permian brachiopod fauna and Gondwana-South-East Asia relationships

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The island of New Guinea, geologically and structurally part of the Indian-Australian plate^{1,2}, is shown on continental reconstructions of the Permian globe as forming the northeastern part of the supercontinent of Gondwana^{3,4}, facing a large Tethys Ocean to the north. The existence of an ocean separating South-East Asia from Gondwana by some 45° of latitude during the Permian has been widely disputed because of the strati-graphical, structural⁵⁻¹⁰ and palaeontological¹¹⁻¹⁴ links between the regions. Palaeontological comparisons of New Guinea and Asia are essential for testing whether or not South-East Asia was a part of Gondwana during the Permian^{15,16}. We now give an account of the first, diverse, reliably dated, Permian articulate brachiopod fauna to be discovered from the island of New Guinea. This fauna is interpreted as reflecting the geographical proximity of Thailand and Irlan Jaya during the late early Permian.

Reports of Permian marine faunas from Papua New Guinea^{17,18} have been shown to be erroneous¹⁹, the faunas being Triassic in age. Previous collections of late Palaeozoic marine faunas from Irian Jaya have been inadequate for precise dating and have provided only a generalized Permo-Carboniferous age¹. The new collections, from the Birds Head region of Western Irian Jaya, are from four localities within the Aifat Formation of the Aifam Group, outcropping on the Taminabuan 1:250,000 sheet area²⁰. The Aifat Formation is some 1,100 m thick and consists chiefly of dark calcareous shale and marl with limestone intercalations. Three of the localities have brachiopod faunas which are remarkably similar to those from the Rat Buri Limestone of Thailand^{20,21} of late

Baigendzhinian (latest Artinskian)21 or early Kungurian22 age. They also have some links with brachiopod faunas of Western Australia and Bitauni, Timor. The fourth locality has yielded a fauna, sharing no species or genera in common with the other three localities, which contains a species of the rare chonetacean Quinquenella that is morphologically close to a Western Australian species23 of the genus. This comparison is used to date the Irian Jaya species as late Baigendzhinian. The locality, in addition to Quinquenella, has only yielded fragmentary spiriferid brachiopods which are unidentifiable at this stage. Of the 14 genera from the Irian Jaya fauna, 13 are present in the Rat Buri Limestone faunas; 11 species are morphologically close between the two regions (Table 1).

Most of the classic Permian brachiopod faunas of Timor, such as the Basleo fauna, are much younger than the Irian Jaya and Thailand faunas21. An exception is the Bitauni fauna which exhibits some similarities with the Irian Jaya fauna (such as representatives of Stereochia and Stenoscisma close to Stenoscisma sp. nov.) and the Thailand and Western Australian late early Permian faunas²¹ (such as the presence of Retimarginifera). However, the Bitauni fauna has not been fully described thus preventing detailed comparison. Furthermore, the tectonic setting of the Bitauni fauna is confused because the Timor limestones occur as lenses in the Tertiary Bobonaro Scaly Clay Olistostrome. They have been interpreted as being allochthonous tropical limestones from the northern margin of Tethys24. However, limestone is known from the Australian north-west offshore shelf2 and the Timor limestone faunas are subtropical, not tropical, and show greatest taxonomic affinities to the faunas of northern India and north-west Australia2. The Timor limestones are probably of northern Gondwanan affinity². Links between the late early Permian brachiopod faunas of Thailand and Western Australia, although previously considered to be minimal25, are becoming more apparent as the faunas of Western Australia become better known26. Similarities between Western Australian and South-East Asian Permian molluscan faunas are also significant²⁷

A Permian geographical position for Thailand close to that of Irian Jaya and hence to the northern margin of Gondwana is a possible explanation of these faunal links and the mixed Cathaysian-Glossopteris Permian flora from Thailand that has previously been explained by parallel evolution28. The mixed Permian floras of Thailand and Irian Jaya1 were apparently on the northern fringe of the Gondwana Glossopteris Floral Realm. The Irian Jaya Glossopteris occurs in stratigraphically concordant sediments above the Aifat Formation. The source of the Permian rock sequence of the Birds Head Region is most probably the large area of basement (Kemum Formation) exposed to the north of the outcrops of Permian rocks. The Kemum Formation of Silurian age, is an autochthonous

Table 1 Late early Permian brachiopods from Irian Jaya²⁰ comparison with those from the Rat Buri Limestone

Irian Jaya	Thailand
Streptorhynchus sp.	Streptorhynchus khwaense
Rhipidomella	Present*
Chonetinella sp. nov.	Present*
Stictozoster sp. cf. S. leptus	Stictozoster leptus
Stereochia sp. nov.	Stereochia litostyla
Linoproductus sp. nov.	Linoproductus sp. indet.
Cancrinella sp.	Present*
Stenoscisma sp. nov.	Stenoscisma sp. A
Stenoscisma sp. cf. S. tetricum	Stenoscisma tetricum
Cruricella sp.	Cruricella couria
Callispirina sp.	Callispirina austrina
Spiriferellina sp.	Spiriferellina yanagidai
Hustedia sp. cf. H. ratburiensis	Hustedia ratburiensis
Cleiothyridina sp.	Cleiothyridina tribulosa
Quinquenella sp. nov.	

^{*}The genus is present in the Thailand faunas but the species is dissimilar to the Irian Jaya species.

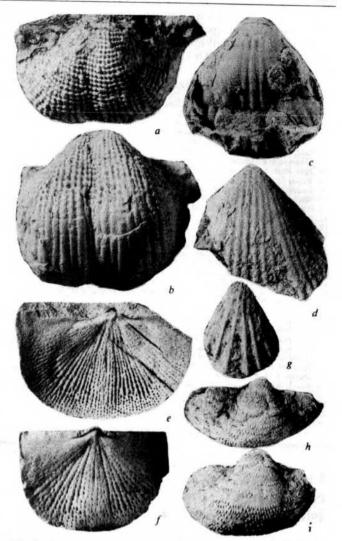


Fig. 1 Permian brachiopods from Irian Jaya. a, b, Ventral posterior and ventral views of Stereochia sp. nov., ×1. c, Ventral view of Stenoscisma sp. nov., ×2. d, Ventral view of Stenoscisma sp. cf. S. tetricum Grant 1976, ×2; e and f are views of two internal moulds of dorsal valves of Quinquenella sp. nov., ×4.5 and ×5 respectively; g is a ventral view of Hustedia sp. cf. H. ratburiensis Waterhouse & Piyasin 1970, $\times 2$; h and i are ventral posterior and ventral views of Stictozoster sp. cf. S. leptus Grant 1976, x2.

sequence within the Birds Head Region. A palaeomagnetic investigation of the Birds Head Region is being undertaken by the Bureau of Mineral Resources, Geology and Geophysics.

The new palaeontological evidence presented above supports the incorporation of much of South-East Asia onto the northern margin of Gondwana 10. Permian oceanic Tethys appears to have been north of Southern Thailand, because systematic analysis of the brachiopod fauna has indicated ease of migration between Southern Thailand, Timor, Irian Jaya and Western Australia of closely related species in the late early Permian2 Articulate brachiopods appear to have a free-swimming larval stage of a few hours or days29. This limiting factor, in addition to latitudinal temperature zones and presumed surface currents of the Permian oceans³⁰, would have been a major obstacle to brachiopod migration across a vast Tethys ocean. While several Permian brachiopod genera do exhibit a disjunct distribution, occurring in both Gondwanan and Boreal faunas31, the similarity at the species level between the Irian Jaya and Thailand faunas apparently reflects the geographical proximity of the two regions. The similarity of species seems to imply a relatively narrow latitudinal spread for Irian Jaya and Thailand during the late early Permian, possibly within 5°-10°, because species may range widely along latitudes but they tend not to range far across latitudes32

We thank Dr H. M. S. Hartono, the director, Geological Research and Development Centre, Bandung, Indonesia and the director, Bureau of Mineral Resources, Australia for permission to publish, and Dr S. K. Skwarko for continued advice.

Received 20 November 1981, accepted 5 February 1982

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Lateglacial and early Flandrian chronology of the Isle of Mull, Scotland

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The Isle of Mull, which lies off the west coast of Scotland (Fig. 1), was partially over-ridden by ice from the mainland during the build-up of the late Devensian ice sheet but, in common with the adjacent islands of Rum² and Skye³, supported smaller ice masses, including independent valley and cirque glaciers during the Loch Lomond Stadial^{4,8} (approximately equivalent with the Younger Dryas of Scandinavia^{6,7}). However, although the recent glacial history of Mull is understood, at least in general terms, little is known about the nature and timing of the major environmental changes that preceded and succeeded the Loch Lomond Stadial on the island. We present here pollen-stratigraphical evidence supported by 15 radiocarbon dates which provide new information on landscape changes on the Isle of Mull from the wastage of the last Scottish ice sheet to the early part of the Flandrian period. The radiocarbon dates are of particular significance as they form the basis for the first Lateglacial and early Flandrian chronology for the islands of the Scottish Inner Hebrides.

Four sites have been investigated (Fig. 1). Loch an t-Suidhe (grid ref. NM370215, altitude 30 m OD) on the Ross of Mull and Mishnish (grid ref. NM455565, altitude 120 m OD) to the west of Tobermory are infilled basins which lie outside the area

affected by Loch Lomond Stadial ice, and contain a typical Lateglacial sequence⁸ of organic muds between minerogenic sediments, these deposits in turn being overlain by Flandrian muds and peats. Coire Clachach (grid ref. NM613304, altitude 200 m OD) and Torness (grid ref. NM643333, altitude 110 m OD) are located in Glen More within the mapped limits of Loch Lomond Stadial ice, and these infilled kettle-hole basins contain only Flandrian limnic and terrestrial sediments. At Loch an t-Suidhe and Mishnish, samples for pollen analysis and radiocarbon dating were removed from a deep part of the basins using a piston corer of 5 cm diameter. At the sites in Glen More, however, it was necessary to ensure that the earliest sediments that had accumulated following the wastage of Loch Lomond Stadial ice were being sampled, and thus the infills along a 5 m by 10 m grid pattern were sounded to establish the subsurface contours of the basins9. Cores were then taken from the deepest point. At all the sites, samples for radiocarbon dating were obtained by bulking gyttja material from comparable biostratigraphical horizons in up to six closely-spaced cores, the precise levels from which individual samples were to be taken being determined on the basis of relative pollen counts in each core. In this way, narrow bands of sediment 2-5 cm in thickness could be dated. The radiocarbon dates obtained and the principal characteristics of the dated horizons are presented in Table 1.

Difficulties in the dating of Lateglacial and early Flandrian pollen-stratigraphical records in limnic sediments arise because of the possibility of (1) 'hard water error' particularly in dated samples from newly-deglaciated terrain'; (2) contamination by younger carbon where the sediments have been affected by percolating groundwaters^{12,13}; (3) delayed ice-melt in kettleholes which can produce a basal biostratigraphical record and corresponding radiocarbon dates that postdate regional deglaciation at some sites by hundreds or even thousands of years 14,15; (4) relatively slow rates of sediment accumulation in many basins at times of rapid environmental change which result in poor levels of stratigraphical resolution for the dating of key pollen-assemblage zones¹⁶. There are also uncertainties associated with the radiocarbon method. As many of the potential error sources cannot be quantified at individual sites, it is suggested that the dating of Lateglacial and early Flandrian events within any one area should be based on radiocarbon dates from different biostratigraphical horizons. An indication of the 'true' radiocarbon age of each horizon can then be obtained by taking the mean of the individual values. This procedure is followed where possible for the new radiocarbon dates reported here. In averaging the dates from Mull it is, of course, assumed that no significant time-transgression is associated with the vegetational developments identified at each site, a resonable assumption in view of the relatively small size of the island.

Figure 2 shows the dates listed in Table 1 plotted with one standard deviation about the mean in relation to the principal biostratigraphical and lithostratigraphical horizons. The evidence suggests the following chronostratigraphical framework for environmental changes:

(1) Corylus rise: two dates obtained for this event show remarkably close agreement, averaging ~8,800 yr BP. Radiocarbon dates on the Corylus expansion at sites on the Isle of Skye range from 9,655 to 7,500 yr BP, although the latter age determination was considered to be aberrant 17,18. However, comparable dates to these from Mull have been obtained from sites in Wester Ross $(8,951\pm120 \text{ yr BP}^{19})$ and the Spey Valley $(8,670\pm150 \text{ yr BP})^{20}$. The available evidence points to the widespread colonization by hazel of the Scottish Highlands and the islands of the Inner Hebrides shortly after 9,000 yr BP. The dates from Skye might indicate local exceptions to this generalization, but this requires further examination. The expansion of hazel on Mull appears to postdate that in lowland Scotland by some 300-400 yr (ref. 21).

(2) Juniperus maximum and Betula expansion: the two dates obtained for the early Flandrian Juniperus maximum average \sim 9,600 yr BP, while the single date obtained for the Betula expansion (9,350±70 yr BP) is in good stratigraphical agreement with the former and also with the dates for the later

Corylus rise.

(3) Loch Lomond Stadial-early Flandrian transition: biostratigraphical resolution of the Loch Lomond Stadial-early Flandrian transition in Scottish sites is seldom clear because of the often slow rates of sediment accumulation, and the rapid vegetational changes that occurred between climatic amelioration at the close of the Loch Lomond Stadial and the expansion of juniper scrub. In addition, it is often impossible to develop a chronology based on radiocarbon which differentiates between the individual pollen-stratigraphical phases within this time period. The dates from Mull on all pre-Juniperus horizons above the Loch Lomond Stadial-early Flandrian lithostratigraphical boundary have, therefore, been grouped together. Of the six dates shown in Fig. 2, that from Coire Clachach $(9,530 \pm 80 \text{ BP})$ is clearly aberrant. The others range between $10,000 \pm 70 \text{ yr}$ BP and 10,440 ± 80 yr BP suggesting a mean age of 10,200 yr BP for the establishment of more stable conditions on Mull following the harsh environment of the Loch Lomond Stadial. The dates from Loch an t-Suidhe are considered to be of particular significance. Sedimentation in this basin seems to have been unusually rapid during the Loch Lomond Stadialearly Flandrian transition, and it was possible to obtain three radiocarbon dates (SRR 1800-1802) from biostratigraphical horizons preceding the Juniperus phase. The fact that these dates are closely grouped, internally consistent and in broad agreement with a number of dates from basal Flandrian sediments from other Scottish sites^{22,23} reinforces the suggestion^{24,25} that the transition to more stable environmental conditions in Scotland at the end of the Loch Lomond Stadial occurred well before 10,000 yr BP.

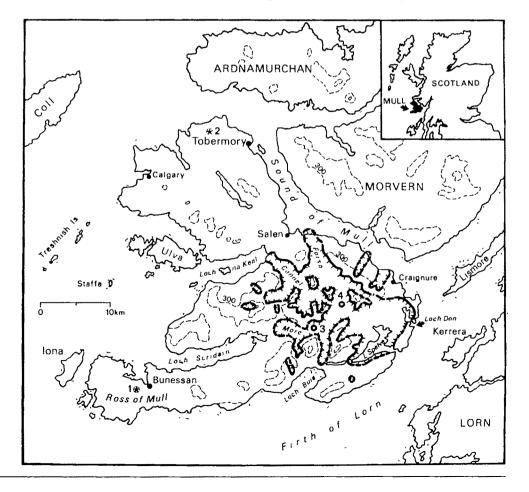
(4) Close of the Lateglacial Interstadial: this event is dated on the Isle of Mull to ~10,700 yr BP on the basis of two age determinations which are in remarkably close agreement. The date is younger than the age assigned to the lower boundary

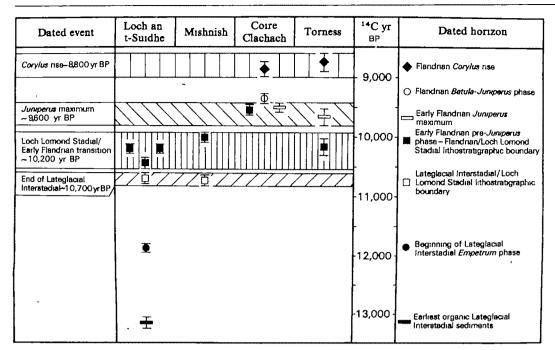
Table 1 Radiocarbon dates from the Isle of Mull

Site	Sample no.	Depth (m)	Thickness (cm)	Date (yr BP)
Loch an t-Suidhe	SRR-1800	7.74	5	$10,200 \pm 70$
Loch an t-Suidhe	SRR-1801	7.80	5	$10,200 \pm 70$
Loch an t-Suidhe	SRR-1802	7.85	5	$10,440 \pm 80$
Loch an t-Suidhe	SRR-1803	8.36	5 .	$10,690 \pm 70$
Loch an t-Suidhe	SRR-1804	8.63	5	$11,860 \pm 80$
Loch an t-Suidhe	SRR-1805	8 77	5	$13,140 \pm 100$
Mishnish	SRR-1806	4.96	' 4	$10,000 \pm 70$
Mishnish	SRR-1807	5.08	4	$10,730 \pm 60$
Coire Clachach	SRR-1594	5.80	5	$8,870 \pm 120$
Coire Clachach	SRR-1595	6.00	5	9.350 ± 70
Coire Clachach	SRR-1596	6.65	5	$9,500 \pm 70$
Coire Clachach	SRR-1597	6.70	5	$9,530 \pm 80$
Torness	SRR-1799	6.65	•2	$8,760 \pm 140$
Torness	SRR-1798	6.67	2	$9,660 \pm 140$
Torness	SRR-1797	6.69	2	$10,170 \pm 150$

of the Younger Dryas chronozone in north-west Europe⁶, and is also younger than age determinations on comparable horizons at several sites on the Scottish mainland 18,20,26. One possibility is that this reflects the more westerly location of Mull and its exposure to moderating maritime influences which could have produced a delayed geomorphological and vegetational response to climatic deterioration, the effects of which were reflected earlier in the pollen stratigraphy at sites on the Scottish mainland. However, it is now generally accepted that climatic deterioration at the onset of the Loch Lomond Stadial was a result of oceanographic changes in the North Atlantic 27,28, in particular the southward migration of the oceanic (and atmospheric) polar front, and therefore climatic deterioration might be expected earlier in the west and not later as is implied by the radiocarbon dates. Yet, at other sites in eastern and central Scotland, dates have been obtained from Lateglacial profiles which are comparable with those reported here from

Fig. 1 The Isle of Mull. Pollen sites: 1, Loch an t-Suidhe; 2, Mishnish; 3, Coire Clachach; 4, Torness. The heavy lines show the limits of Loch Lomond Stadial ice (after ref. 5). Contour interval 300 m.





Flg. 2 Radiocarbon dating of Lateglacial and early Flandrian environmental changes at the four sites on the Isle of Mull.

Mull 18,29,30. Clearly, therefore, there is no consistent spatial pattern in Scottish radiocarbon dates for the close of the Late glacial Interstadial, and it may be that local site factors rather than the macroscale climatic changes were of greater importance in determining the precise nature of lake basin biostratigraphy at the Lateglacial Interstadial-Loch Lomond Stadial transition.

(5) Lateglacial Interstadial: two dates were obtained from the Lateglacial Interstadial deposits at Loch an t-Suidhe. The immigration of Empetrum and, to a lesser extent, Juniperus and Betula, was dated at 11,860 ± 80 yr BP, while the basal organic sediments yielded a radiocarbon age of 13,140 ± 100 yr BP. A comparison between the three dates from the Lateglacial Interstadial at Loch an t-Suidhe (SSR 1803-1805) in relation to their position in the stratigraphical column (Table 1) indicates that the rate of sediment accumulation in the basin during the later part of the Interstadial was almost double what it had been in the earlier stages. Although sedimentation in lakes can be highly variable as a result, for instance, of different rates of inwash and of organic productivity, such a marked discrepancy is unusual, and may reflect errors in one or more of the radiocarbon dates. The age determination of $11,860 \pm 80 \text{ yr BP}$ is difficult to evaluate in the context of the Scottish Lateglacial because of the marked contrasts which appear to have existed in vegetational developments between Mull and the mainland, particularly in respect of the reduced representation of woody taxa in the former area. Moreover, very few dates have so far been published on vegetational changes during the Lateglacial Interstadial in Scotland. The pollen spectra associated with the basal date (SRR 1805) are characterized by a Rumex-Salix-Gramineae-Cyperaceae assemblage and reflect an early stage in plant colonization following the disappearance of the Devensian ice sheet. Although a hardwater error cannot be excluded11, the date of 13,140±100 yr BP is very similar to age determinations on comparable biostratigraphical horizons at sites on the Scottish mainland 18,30-33, and is also in agreement with the inferred date for the replacement of polar by warmer waters around the coasts of western Britain²⁷. If correct, therefore, it would support the suggestion that climatic amelioration at the beginning of the Lateglacial Interstadial in Scotland began around, or even before, 13,000 yr BP (ref. 34).

We conclude with the following framework for environmental change. Wastage of the Scottish ice sheet was followed by climatic amelioration, perhaps around 13,000 yr BP, after which Mull gradually developed a vegetation of Empetrum heath, juniper and birch scrub and open grassland. Climatic deterioration at, or before 10,700 yr BP led to the breakup of the Interstadial vegetation cover, the development of a local ice cap and several separate smaller glaciers in the hills of central Mull, and the establishment of severe periglacial conditions throughout the island. Climatic amelioration around 10,200 yr BP caused the final wastage of the Loch Lomond Stadial glaciers and initiated the early Flandrian plant succession. This began with an Empetrum phase and was succeeded by Juniperus expansion between 9,600 and 9,500 yr BP, the establishment of open birchwoods by ~9,300 yr BP and the immigration of Corylus around 8,800 yr BP. The above chronology is strengthened by the internal consistency of the Mull dates and by the broad measure of agreement between dates from similar biostratigraphical horizons, although clearly the precise timing of events during the early Lateglacial Interstadial remains enigmatic.

This research was financed by the NERC, by the Pantyfedwen Fund of St David's University College, Lampeter, and by the City of London Polytechnic. We thank Dr D. D. Harkness for the radiocarbon dates, Dr R. Cornish, Dr A. G. Dawson, Dr. J. M. Gray and Mr. D. John for assistance with the fieldwork.

Received 5 November 1981, accepted 5 February 1982

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Life span of the biosphere

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There has been life on Earth for at least 3,500 Myr but the assumption that a comparable future lies ahead may not be justified. Main sequence stars appear to increase their burning rate as they age. Thus the Sun, if a typical star, can be predicted to have increased its output by 30% since the Earth's origin 4.500 Myr ago¹. The maintainance of an equable climate since life began probably required some means of planetary thermostasis. The Gaia hypothesis proposed by Loyelock and Margulis² included an unspecified biological means for climate control. Walker et al.3 suggests an abiological automatic thermostasis in which the atmospheric abundance of CO2, a greenhouse gas, adjusts to resist the warming tendency of the increased solar flux. Here we discuss possible links between the biological and geological control mechanisms. It is clear that whatever the mechanism, atmospheric CO2 is now close to its lower limit of partial pressure, so the biosphere may soon, in geological terms, be exposed without protection to the predicted progressive increase of solar luminosity.

In spite of the probability of a 25% increase in solar luminosity it is reasonably certain that the Earth's climate has undergone little change since life began. Both the geological record and the persistence of life indicate that neither global freezing nor boiling conditions have ever prevailed. Indeed, the Earth's mean surface temperature has probably never departed from the range 5-50 °C and, contrary to the predictions of simple physics, may have been warmest at the beginning when the Sun was small and has cooled ever since.

Several models have been proposed to account for the Earth's relatively constant temperature. Sagan and Mullen's were the first to suggest that the infant biosphere was warmed by an atmospheric gas which exerted a 'greenhouse' effect by transmitting sunlight while hindering the escape of heat to space.

Although water vapour makes the most significant contribution to the greenhouse effect in the contemporary atmosphere. because of its physical properties water is an unlikely candidate for long-term thermostasis. Its relatively high freezing and boiling points render its blanketing effect prone to positive feedback in the face of extreme temperature excursions. For example, a sudden fall in temperature could result in an increase in the size of the polar ice caps and the seasonal snow fields (thus increasing the efficiency with which radiation is reflected from the Earth) and a corresponding fall in the atmospheric humidity. Both effects could contribute to a further drop in temperature. On the other hand, a sudden rise in temperature would increase the water vapour content of the atmosphere, which would in turn push the temperature still higher. In consequence, the complexities of cloud cover feedback effects over

long periods of time have focused attention on simpler atmospheric models involving other greenhouse gases³⁻⁵

Sagan and Mullen⁵ suggested that the early atmosphere was rich in ammonia and other reduced gases and that these provided the blanket which kept the Earth sufficiently warm for life to emerge. Relatively high partial pressures of ammonia were also seen as essential for the chemical evolution of life and, as ammonia is rapidly photolysed in the atmosphere, a significant inorganic source was sought⁶. Recent work, however, suggests that high partial pressures of ammonia are not essential for the abiological production of amino acids⁷ and that the primordial atmosphere probably contained little ammonia but relatively high partial pressures of CO28. The concept of an early Earth warmed by the blanketing effect of its atmosphere is still therefore feasible but with CO₂ the preferred blanket gas^{4,7}. According to this hypothesis whatever greenhouse gas or other agency kept the young planet warm, it must have been smoothly and actively reduced from then until now; otherwise the mean temperature would have progressively increased with increasing solar heat flux and might by now have exceeded 50 °C, the critical upper limit for most life.

Walker et al.3 have proposed that the climate could have been controlled solely by abiological negative feedback involving a gradual decline in the atmospheric partial pressure of CO₂ in response to a continuous increase of the Sun's luminosity. Briefly, an increase in temperature accelerates the rate of reaction between CO₂ and calcium silicate rock. The input of CO₂ from tectonic sources is assumed to be constant so that an increased heat flux leads to increased weathering of the rock, a faster removal of CO_2 and hence a lower atmospheric CO_2 partial pressure. This cybernetic process acts to resist the rise in temperature which would otherwise result from the increase in solar luminosity.

This abiological mechanism could have operated early in the Earth's history but the resulting climatic control would have been easily perturbed by fluctuations in the volcanogenic flux of CO₂. These effects could have been exaggerated by the positive feedback loops introduced by the ocean-atmosphere interaction. As the solubility of CO₂ in water decreases with increasing temperature, an atmospheric (and hence oceanic) warming would result in a release of CO₂ to the atmosphere which would produce a further temperature rise. A corresponding positive feedback would occur on cooling. At present geological input and removal constitute only a few tenths of a per cent of the biologically driven fluxes¹⁰ and so it is instructive to see how the biological and geological mechanisms can be

There is little doubt that current weathering of silicate rocks is biologically, not geochemically, determined. The partial

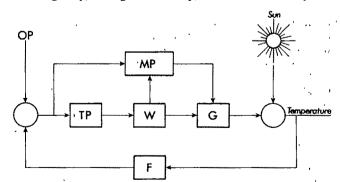


Fig. 1 A feedback process in which the productivity of the terrestrial biosphere (TP) or the marine biosphere (MP) influences the greenhouse effect (G) and hence the global mean temperature via control of the atmospheric partial pressure of carbon dioxide. These biological processes modulate the geological control exerted by the weathering process (W) against a constant input from tectonic sources. The loop is closed by the feedback process (F, see text) which transduces from the mean temperature a productivity that can be compared with the operating productivity (OP) of the biosphere.

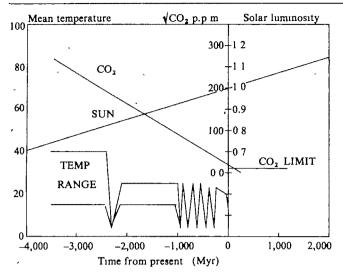


Fig. 2 Evolution of the climate showing the variation of solar luminosity, relative to its present value taken as 1.0. Also illustrated on the same time scale are the proposed decline in carbon dioxide concentration, expressed as the square root of the concentration in parts per million (p.p.m.) by volume and the approximate range of mean surface temperatures in °C (see text).

pressure of CO₂ in the soil where the chemical weathering takes place is 10-40 times higher than the atmospheric level¹¹. These high partial pressures are maintained by the biological oxidation of organic detritus and the rate of oxidation approximately doubles for each 10 °C rise in temperature. The biota seem to act as a sensor of temperature change and as an amplifier to magnify the rate of weathering of the silicate rocks and pumping of CO₂ from the air. The present geochemical balance for CO₂ between input from tectonic sources and removal by weathering¹⁰ requires a partial pressure in the soil of nearly 10 mbar.

The direct involvement of the biota in weathering would only be possible after the evolution of a land microfauna and flora and the establishment of viable soil communities. Although such communities probably predate the invasion of land as deduced from fossils, it is possible that life was largely confined to the sea during the Archaean. Once communities based on photosynthetic carbon fixation were established in the oceans, however, they could amplify indirectly the geochemical feedback. In the contemporary ocean the biota limit the atmospheric partial pressure of CO₂ by maintaining a continuous flux of particulate organic matter into the deep ocean¹². This flux results from primary production in the surface layers which is limited by the rate of supply of nutrients, notably nitrogen and phosphorus, from riverine inputs and from the slow recirculation of nutrient- and carbon dioxide-rich deep-ocean water. If, as suggested by the geological model³, the rate of rock weathering increases due to a rise in global temperature, then the rate of supply of nutrient elements to the oceans will also increase. This will enhance primary productivity and further reduce atmospheric CO2 levels. If, on the other hand, the temperature falls the nutrient supply will diminish, productivity will decrease and excess CO2 will be released from the recirculating deepocean water into the atmosphere, thus encouraging a temperature rise¹². Here, too, the biota can use geologically driven processes to provide additional temperature stabilization. If life ceased now, the new geochemical balance would require an increase of atmospheric CO2 and hence temperature until removal by weathering again equalled the input of CO₂ from volcanoes. The present low atmospheric CO2 pressure and relatively low temperature are therefore a direct consequence of life. This is consistent with the Gaia hypothesis² which suggests that evolutionary pressures ensure that biologically driven processes which influence the chemical composition of the atmosphere and hence the climate so as to keep the environment optimal for the biosphere will have been preferentially selected.

Figure 1 illustrates a highly simplified model of the combined biological and geological regulation of temperature. As the temperature falls, CO_2 fixation by photosynthesis and chemical weathering declines until at $\sim\!0\,^\circ\mathrm{C}$ it nearly ceases. CO_2 input from tectonic sources continues and the atmospheric partial pressure of CO_2 will rise. Through the greenhouse effect, this increase resists any further decline in temperature. In a similar way high temperatures and high CO_2 pressures are both conductive to rapid weathering which serves to resist a continuing rise in temperature. Thus the mean surface temperature will tend to a value optimal for the contemporary biota within the other constraints of its planetary environment.

Figure 2 illustrates the probable solar output from 4,000 Myr ago to 2,000 Myr hence and gives on estimate of the corresponding global mean temperature and CO₂ partial pressure. Solar luminosity is predicted to increase, according to Newkirk¹. An alternative analytical expression for the time variation of solar luminosity¹³ might be useful for more detailed calculations.

The range of past temperatures is not accurately known. That shown in Fig. 2 is our estimate of the probable limits of palaeoclimates and it is based on the following assumptions. (1) During the Archaean the biosphere was wholly prokaryotic. As the upper temperature limit for contemporary prokaryotes is 50 °C, the upper limit for the Earth's mean temperature was set lower, at 40 °C, to allow for zonal and other variations. No glaciations are known during this period 14,15 so the lower limit of temperature is set at 15 °C, close to the present value. (2) -2,200 Myr marks the end of the Archaean and the first extensive glaciation^{14,15}, as well as the appearance of free oxygen. Temperatures might have risen after this glaciation to Archaean levels or they may have been similar to the levels now thought characteristic of the interglacial periods—20-30 °C. We chose the latter estimate as the upper limit from -2.2 Gyr until the present. The lower limit is set by the physical properties of water. There is no indication that total freezing took place, so a mean temperature of 0 °C or below is very unlikely. Glaciations^{14,15} are associated with mean temperatures of 5-10°C and interglacials with lower limits perhaps 5-15 °C higher. The time scale of the figure is so compressed that the present interglacial climate cannot be resolved from the low values of the last glaciation 10,000 yr ago.

The other quantity illustrated in Fig. 2 is the CO_2 pressure needed to compensate for the changing solar luminosity. This estimate is based on a partial pressure 3,500 Myr ago of 7,000 p.p.m. (refs 4, 17). It is linked to the present value of 320 p.p.m. on the assumption of a linear relationship between the square root of the CO_2 pressure and the solar luminosity for a constant mean temperature. The exact relationship may be different, however, and an analysis of the geological mechanism³ suggests that a 2/3 power relationship might be more appropriate. Nonetheless, such differences are unlikely to affect the qualitative conclusions that can be drawn from Fig. 2.

If the principal agent for temperature regulation is the partial pressure of CO2 in the atmosphere and even if the solar luminosity is constant, it seems that we are very close to the lower limit of possible adjustment; also there are no other gases in the air which could serve significantly in this way by the reduction of their partial pressures. Other mechanisms such as an increase in albedo by desert cover might sustain an equable climate but as observed by Henderson-Sellers 16, this is generally neutralized by a concomitant change in cloud cover. On the other hand, if we accept the predicted increase in luminosity and assume that the future decline of CO₂ matches the solar output as may have been the case in the past, then 150 p.p.m. pressure will be reached in about 100 Myr. This concentration is the lower limit tolerable for photosynthesis. Some adaptation to lower CO₂ concentrations and to higher temperatures is possible but it would not buy much time. In human terms the crisis is still infinitely distant but in terms of the life span of the biosphere,

rich with familiar metazoans, we might forecast an end to the long spell of cool and favourable climate.

We thank Dr A. F. Tuck and Professor R. M. Garrels for stimulating suggestions.

Received 4 June 1981, accepted 12 February 1982.

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LDH-B genotype-specific hatching times of Fundulus heteroclitus embryos

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The evolutionary significance of protein polymorphisms has long been debated. Exponents of the balanced theory advocate that selection operates to maintain polymorphisms, while those of the neoclassical school argue that most genetic variability is selectively neutral1. As the 'neutralist' hypothesis implies that allelic isozymes are functionally equivalent, some investigators have examined the biochemistry of protein polymorphisms² while others have concentrated on life history correlates 16-23. Few studies, however, have established that in vitro functional differences are reflected at the whole organism level^{5,21-23}, yet this is a critical link in understanding the significance of protein polymorphisms. We have studied the effects of the kinetically different lactate dehydrogenase-B (LDH-B) allelic isozymes on the rate of development and physiological performance of the fish, Fundulus heteroclitus, and report here that hatching time is highly correlated with LDH-B genotype: LDH-B'B' individuals hatch before LDH-BbB fish while heterozygote (LDH-BaBb) hatching is intermediate. The basis for this phenomenon may be a differential ability to deliver oxygen to respiring tissues. As precisely timed hatching is critical to the survival of Fundulus²⁴, such differences in hatching time between LDH-B genotypes may be an important component of the species' evolutionary strategy.

The LDH-B polymorphism in F. heteroclitus is due to two co-dominant alleles 25,26 which exhibit a cline in gene frequencies along the Atlantic Coast of the United States^{4,27}. We have shown that there are temperature- and pH-dependent kinetic differences between the LDH-B allelic isozymes⁷. Moreover, intra-erythrocyte ATP differences among individuals are correlated with LDH-B phenotype³. The role of ATP as an allosteric modifier of Fundulus haemoglobin is similar to that of 2,3diphosphoglycerate in human red blood cells, in that it decreases the affinity of haemoglobin for oxygen^{28,29}. Thus, fish with the highest levels of intra-erythrocyte ATP (that is, LDH-Bbb) have the lowest blood oxygen affinity³. As respiratory stress has been shown^{30,31} to trigger the hatching mechanism in Fundulus, we hypothesized that the hatching times of LDH-B genotypes should differ because of differences in blood oxygen affinity. Specifically, LDH-B*B* fish should hatch before LDH- B^bB^b individuals.

As an initial test of this hypothesis, fertilized eggs were obtained by stripping gametes from males and females that were randomly paired. Each of the resulting clutches was incubated in a separate Petri dish at 20 °C, pH 7.0 and 15% salinity (Instant Ocean). The incubation medium was changed daily and the fry were collected as they hatched. The LDH-B genotypes of each parental pair and their offspring were determined by gel electrophoresis 26,27 (see Fig. 1). Clearly, hatching of LDH-BaBa eggs was predominant in the first 3 days, while $LDH-B^bB^b$ eggs predominated in the last 3 days. The overall mean hatching times were 11.9, 12.4 and 12.8 days for the LDH-BaBa, LDH-BaBb and LDH-BbBb genotypes, respectively. Individual crosses in which there were more than one genotype also showed that LDH-BaBa eggs hatched before $LDH-B^aB^b$, which in turn hatched before $LDH-B^bB^b$ (Fig. 1).

In addition to establishing an ordered hatching of LDH-B genotypes, our analysis (Fig. 1) also indicated variations between crosses that were not due to the LDH-B locus. If these variations were due to other loci, then pooling gametes from many individuals before fertilization should dramatically reduce the variation between crosses. To test this hypothesis, we performed experiments in which adult fish were segregated by LDH-B genotype into breeding stocks²⁶. Specific LDH-B crosses were made by pooling gametes before fertilization. Consistent with our expectation, these experiments (Tables 1 and 2) almost eliminated variation between similar crosses. Thus, some of the variation among the 20 random crosses in Fig. 1 must have been due to contributions from other loci. On the other hand, Tables 1 and 2 indicate that there are still highly significant differences (P < 0.01) in hatching time between LDH-B genotypes. These data (Tables 1 and 2), together with

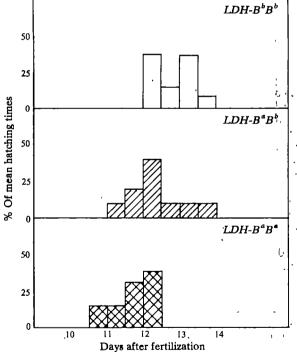


Fig. 1 Distribution of mean hatching times for three LDH-B genotypes from 20 random crosses. Analysis of variance³⁶ indicated significant differences between the crosses (P < 0.05); Duncan's multiple range test³⁶ showed that some of this variation was due to LDH-B genotype (for example, all the LDH-BaBa× $LDHB^aB^a$ crosses hatched before all the $LDH-B^bB^b \times LDH-B^bB^b$ crosses; P < 0.05). There were also significant differences, however, between similar crosses (for example, two of the LDH- $B^aB^a \times LDH$ - B^aB^a crosses hatched sooner than three other crosses of the same type; P < 0.05). The histogram shows the distribution of mean hatching times among genotypes irrespective of parental cross. Separate analyses of each cross in which there were offspring of more than one genotype, showed significant differences (P < 0.05) in hatching time between genotypes in each case.

Table 1 Mean hatching times (days) for each offspring genotype resulting from four LDH- $B^aB^b \times LDH$ - B^aB^b 'mass' crosses

4		Off	spring genoty	pe*
Replicate	N	LDH-B ^a B ^a	$LDH-B^aB^b$	$LDH-B^bB^b$
1	2,142	12.0	12.8	12.9
2	1,273	11.9	12.0	13.1
3	1,396	12.4	12.8	14.8
4	1,051	12.5	12.5	14.3

Sperm and eggs from 40 LDH-BaB males and 40 LDH-BaB females were pooled in separate dishes and then mixed to make each cross.

N, total number of offspring from each cross.

*The data were analysed by a two-way analysis of variance of unweighted means³⁶, which provides a conservative estimate of the variance between genotypes. The differences between genotypes was found to be significant (P < 0.01); there were also significant differences between replicates (P < 0.05) but this estimate is not conservative because the MS_{error} term had to be calculated separately.

the consistent trend from the 20 random crosses (Fig. 1), demonstrate that hatching in F. heteroclitus occurs generally in the following order: LDH-BB phenotype before LDH-BB, then LDH-BbBb.

The eggs of F. heteroclitus are laid in empty mussel shells³² between the leaves of the marsh grass, Spartina alterniflora²⁴, or in the sand above the mean high tide³³. In these conditions, the eggs incubate in air for most of their developmental period. Hatching occurs when eggs laid at one spring tide are immersed in water by the following spring tide. As water covers the eggs, there is a drop in environmental oxygen at the egg surface which is the hatching cue for the embryo³⁰. Therefore, overall plasticity in hatching times may be important in protecting F. heteroclitus populations that live in variable environmental conditions. Our data suggest that premature hatching cues (for example, rainstorms) would select largely against LDH-BBB individuals, while late hatching (that is, after the tide has retreated) would select primarily against the LDH-Bbb phenotype. This argument is particularly compelling in view of the finding of Meredith and Lotrich³⁴ that the mortality of F. heteroclitus in age-class 0 (eggs to fry of 59 mm) is >99.5%.

As respiratory stress initiates hatching and blood oxygen affinity is correlated with LDH-B genotype via intra-erythrocyte ATP concentrations, the simplest interpretation of our data is that the differences in hatching times result from functional differences between LDH-B allelic isozymes. However, our results do not exclude the possibility that other genes, tightly linked to the LDH-B locus, may be responsible for the observed effects on hatching time. Whatever the molecular basis for these differences may be, it is clear that genetic variability at the LDH-B locus (or a closely linked one) leads to a 'developmental polymorphism'35. Determination of the evolutionary significance of this whole organism phenomenon and its underlying molecular mechanisms should help resolve

Table 2 Mean hatching times (days) for offspring from $LDH-B^aB^a \times$ $LDH-B^aB^a$ and $LDH-B^bB^b \times LDH-B^bB^b$ 'mass' crosses

			Offspring g	genotype*			
	Replicate	LDI	H - B^aB^a	LDH - B^bB^b			
	1	11.5	(2,782)	13.0	(2,511)		
•	2	12.5	(2,826)	13.8	(2,654)		
	3	12.7	(3,273)	14.3	(2,716)		

Sperm and eggs from ~40 males and 37-56 females were pooled in separate dishes and then mixed to make each cross. Numbers in paren-

*The data were analysed by a single classification analysis of variance followed by Duncan's multiple range test³⁶. The three LDH- $B^{a}B^{a} \times LDH$ - $B^{b}B^{b} \times LDH$ - $B^{b}B^{b}$ crosses were significantly different from the three LDH- $B^{b}B^{b} \times LDH$ - $B^{b}B^{c}$ crosses (P < 0.05). There were no significant differences between similar crosses.

the controversy between exponents of the 'selectionist' and 'neutralist' schools of thought.

We thank Dr Allyn Kimble for statistical advice, Judith A. DiMichele for technical assistance and Dianne Powers for artistic assistance. This work was supported by NSF grant DEB-79-12216 to D.A.P. and a NIH postdoctoral fellowship (5-F32-GM7889-02) to L.D. This is contribution 1137 from the Department of Biology.

Received 25 August 1981, accepted 24 February 1982

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Local application of retinoic acid

to the limb bond mimics the action of the polarizing region

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The polarizing region, a small group of cells at the posterior margin of the limb bud, acts as a signalling region to specify the pattern of structures which develop across the antero-posterior axis of the limb¹. When a polarizing region is grafted to the anterior margin of a second limb, a mirror-image symmetrical limb develops^{1,2}. There is indirect evidence that the signal from the polarizing region is a diffusible morphogen³. In an attempt to identify the nature of the putative morphogen, we have developed a method whereby extracts of polarizing region cells and chemicals can be locally applied to the wing bud by being bound to implanted beads and other inert carriers. The chemicals tested included hyaluronidase, dibutyryl cyclic AMP and thalidomide. All experiments have been negative until, at the suggestion of Dr J. Pitts, we tested retinoic acid because of its effects on cell-to cell communication and cell differentiation, and we have now for the first time been able to mimic the action of the polarizing region with a defined chemical.

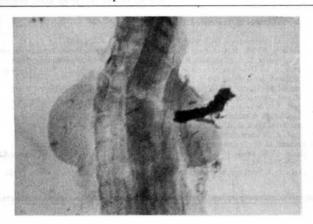


Fig. 1 Dorsal view of part of a fixed embryo to show how the grafts were performed. A strip of retinoic acid-impregnated paper has been placed in an anterior position in the right wing bud.

Small strips of Whatman diethylaminoethyl cellulose (DEAE) paper (~500 µm long and 100 µm wide) were soaked in a range of concentrations of all trans-retinoic acid (Sigma, type XX) dissolved in dimethyl sulphoxide (DMSO; Sigma, grade 1) for 15-30 min. These strips were then grafted wet to slits cut in the anterior margins of wing buds at stages of development before the digits are specified (Hamilton-Hamburger stages 20-21). The slits to which the papers were grafted were cut into the wing at a level opposite somite 16 or the border between somites 16 and 17. Figure 1 shows the type of operation but in this case the paper has been grafted more posteriorly. The embryos were left to develop for a further 6-7 days before they were fixed and the cartilage pattern of the grafted wings examined. The results obtained are presented in Table 1.

DEAE-cellulose papers alone, papers soaked in DMSO (unpublished results of J. McQueen) and papers soaked in the lower concentrations of retinoic acid tested (0.01-2.5 mg ml⁻¹) did not produce additional digits (Fig. 2a). Instead, the most anterior digit, digit 2 and/or the anterior forearm element, the radius, is occasionally absent (digit 2 missing in 2/27 cases, the radius missing in 6/27 cases). However, papers soaked in concentrations of retinoic acid higher than 2.5 mg ml-1 can produce additional digits in the wings of the surviving embryos. We have previously shown that grafts of cells with high polarizing activity lead to the development of a complete duplicate set of digits: 432, in mirror-image symmetry with the normal set, 234, while grafts with progressively lower activities specify a duplicate 3 and 2 and then a duplicate 2 only4.5. The pattern of duplicated digits obtained with retinoic acid suggests that the response to this chemical is similarly dose dependent. With grafts of papers soaked in 5 mg ml⁻¹ retinoic acid, 50% of the wings that develop have duplicate digits and in most cases consist of an additional digit 2 only (Fig. 2b), while with grafts of papers soaked in 7.5 mg ml⁻¹ retinoic acid, nearly every wing that develops has additional digits and furthermore, half of these have duplicated both digits 4 and 3 (Fig. 2c). Duplications were also obtained at concentrations of 10 mg ml⁻¹ using pure retinoic acid (Hoffmann-La Roche). Although the pattern of the production of additional digits mimics the action of the polarizing region, the detailed patterns of cartilage in the duplicated wings, in some cases, at the higher concentrations of retinoic acid resemble those typically produced when signalling is unimpaired but growth is reduced⁶.

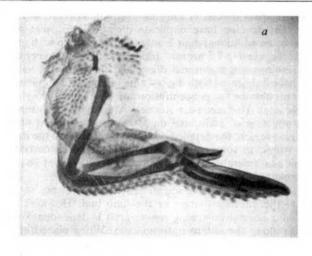
Our assay for polarizing region activity involves placing the graft at the anterior margin of the limb bud. However, the effect of a normal polarizing region graft is dependent on its position along the antero-posterior axis. When placed at the posterior margin it has no effect. We therefore grafted retinoic acid-impregnated papers (10 mg ml⁻¹) to the posterior margin of nine wing buds. Seven gave normal wings, one lacked digits and another gave a tiny extra sliver of cartilage anteriorly. These results are consistent with local application mimicking the polarizing region rather than a systemic effect. Note that the survival of the embryos receiving grafts of the papers soaked in the high concentrations of retinoic acid that lead to the production of additional digits can be reduced (Table 1). Indeed, using two batches of retinoic acid, scarcely any embryos survived that received grafts of papers soaked in retinoic acid at 10 mg ml-1, while with a third batch 100% of the treated embryos survived. Associated with the production of additional digits, embryos that had received grafts of retinoic acid-impregnated paper to the limb bud also showed beak deformities. The beak deformities, which will be described more fully elsewhere, affected the upper beak only and ranged from a complete absence of the upper beak to a slight shortening.

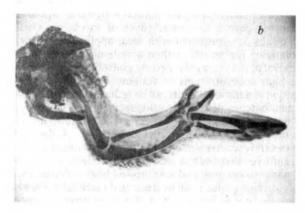
Retinoic acid and vitamin A have a wide variety of effects on cells, for example, inducing differentiation⁷, blocking gap junctions⁸ and affecting glycoprotein synthesis⁹. Added systemically, retinoic acid also has teratological effects on limbs, such as damaging cartilaginous elements¹⁰. It probably acts through a retinoic acid binding protein in the cytoplasm⁹.

Further work is required to determine whether retinoic acid is itself a morphogen or is converted in the limb to such a morphogen; alternatively, it may activate some other system or simply be acting as a weak acid. Moreover, a cautionary note is struck by the fact that the concentration of retinoic acid required to produce extra digits can approach that lethal to the embryo. However, cells soaked in several other toxic chemicals never produced extra digits when grafted to anterior positions in early buds¹¹. These results with retinoic acid also contrast with those studies on neutral induction, where substances producing 'sub-cytolytic' changes trigger neural differentiation without a change in pattern¹². Here, we have the first example

Table 1 The digit pattern in wings that developed following grafts of paper soaked in retinoic acid solutions to anterior positions in early wing buds

					Dig	it pattern		
Concentration of retinoic acid solutions in which papers were soaked (mg ml ⁻¹)	No. of grafts	No. of survivors	Normal 234	2234	3 3 4 or 3 2 3 4	4 3 3 4 or 4 3 4	43234	Other
0 (controls)	6	5	4	4	-	-	-	One 3 4
0.01	6	5	5	_	_	_	_	
0.1	6	6	6	-	-	-	-	
1	6	5	4	-	-	-	-	One 3 4
2.5	10	8	8	_	-	-	-	
5	31	18	9	7	1	1	-	
7.5	16	9	1	3	-	4	-	One 4
10	15	8	1	1	2	1	2	One no digits
Saturated solution	5	0						The second secon





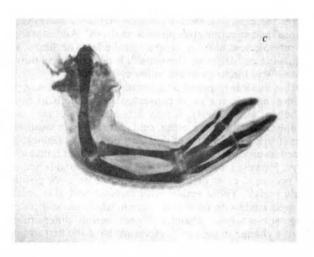


Fig. 2 Dorsal views of whole mounts of wings that developed following grafts of retinoic acid-impregnated strips of paper to anterior positions in the early wing buds. a, Wing that developed following a graft of a strip of paper soaked in 0.1 mg ml-1 acid. The digit pattern is normal: 234. Note that the strip of paper can be seen in this wing and has ended up at the head of the humerus. b, Wing that developed following a graft of a strip of paper soaked in 7.5 mg ml⁻¹ retinoic acid. Digit pattern is 2 2 3 4. c, Wing that developed following a graft of a strip of paper soaked in 7.5 mg ml⁻¹ retinoic acid. Digit pattern is 4 3 3 4.

of a well defined spatial pattern of cellular differentiation being created by a chemical changing positional values in a manner that closely mimics that of the normal signalling tissue.

This work was supported by the MRC and by NIH grant GM23928 to B.A. We thank A. Crawley for help with photography.

Received 30 October 1981: accepted 5 March 1982.

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Long-term complications of virus-induced diabetes mellitus in mice

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In man, the early metabolic abnormalities associated with diabetes mellitus are followed by various long-term complications1. The cause of these complications is not clear, but they may be secondary to persistent abnormalities in blood glucose levels². In mice, several viruses can infect and destroy pancreatic β cells and produce acute-onset insulin-dependent diabetes mellitus (IDDM) characterized by hypoinsulinaemia, hyperglycaemia, glycosuria, polydypsia and polyphagia3. The virus most studied is the M variant of encephalomyocarditis (EMC) virus4.5. Long-term complications, however, have been difficult to demonstrate because in most mice, the diabetes is transient and mild. Recently, by plaque purification, we have shown that the M variant of EMC virus actually has two stable variants: one diabetogenic (designated D) and the other nondiabetogenic (designated B). Further studies revealed that the B variant reduces the severity of the diabetes produced by the D variant6. We now report that when mice are inoculated with the D variant alone, many develop severe and prolonged hyperglycaemia followed by some of the long-term complications of diabetes, including reduced lifespan, glomerulosclerosis and ocular changes.

SJL/J male mice, 5-6 weeks old, were infected with 5×10^5 plaque-forming units (PFU) of the D variant of EMC virus and at various intervals over 6 months, non-fasting blood glucose levels were determined and mortality calculated. As seen in Table 1, 7 days after infection, 85% of the mice were diabetic with a mean blood glucose level of 426 ± 94 mg %. The number of diabetic mice decreased with time and at 150 days, only 12 of the mice were still diabetic with a mean glucose level of 290 ± 44 mg %. In contrast, age-matched uninfected controls showed no elevation of blood glucose levels. The EMC-infected diabetic animals also showed substantially higher mortality than age-matched uninfected controls or infected but non-diabetic mice (Table 1). Between 7 and 30 days after infection, only 7.1% of the diabetic mice died. At each interval thereafter, there was an increase in mortality associated with the duration of diabetes. In contrast, the infected non-diabetic mice, including those that converted from the diabetic to the non-diabetic state, showed low mortality. After 6 months, the cumulative mortality of the infected diabetic mice and uninfected controls was 64% and 17% respectively.

Evidence that the elevated blood glucose levels were responsible for the increased mortality is provided by the fact that, at each of the intervals up to 120 days, the overall mortality of the diabetic mice having blood glucose levels above the median was about three times greater than that of those having blood glucose levels below the median.

In addition to the increased mortality, the diabetic mice showed evidence of long-term complications. At 2 months after infection, light microscopy revealed little or no change in the kidney, but after 4 months, mild to moderate mesangial thickening was found. At 6 months, the changes were quite dramatic: all 12 diabetic mice that were killed at 6 months revealed thickening of the Bowman's capsule, prominent nodular and diffuse glomerulosclerosis (Fig. 1b), an increase in the mesangial matrix and thickening of the tubular and peripheral glomerular basement membranes. Occasional glomeruli had capsular drop lesions, and the afferent and efferent arterioles showed evidence of hyaline sclerosis. In contrast, the glomeruli, tubules and arterioles of 12 age-matched uninfected controls (Fig. 1a) and 3 age-matched infected but non-diabetic mice appeared essentially normal.

Transmission electron microscopy of kidneys from diabetic mice revealed moderate thickening of the peripheral glomerular basement membrane. Approximately 20 glomeruli from each of 12 control and 12 diabetic mice (at 6 months) were examined and 5-20 measurements were made per sample as described previously⁷. The peripheral glomerular basement membrane was 91 ± 3.1 nm thick in uninfected controls (Fig. 1c) and 382 ±

6.2 nm in mice that were diabetic for 6 months (Fig. 1d). The foot processes were fused in some areas but intact in others, and the mesangium was thickened markedly. Uninfected agematched controls showed normal glomerular structure, normal basement membrane and distinct delineation of the glomerular foot processes.

Approximately 10 glomeruli from each of 12 control and 12 diabetic mice (at 6 months) were examined also by scanning electron microscopy. The glomeruli of uninfected mice revealed the normal contour of Bowman's capsule, epithelial cells and foot processes (Fig. 1e), but in diabetic mice, there was effacement of the surface contours (Fig. 1f) and considerable atrophy of the glomeruli, which in some cases occupied only one-third of the Bowman's capsule.

In addition, long-term ocular abnormalities were observed in the same 12 diabetic mice that were examined for kidney lesions. The corneal epithelium of mice that were diabetic for 6 months showed marked irregularity of all layers (Fig. 2b) compared with age-matched uninfected controls (Fig. 2a). In particular, the basal layer and the junctional complexes were focally deficient. The stroma and Descemet's membrane were normal. We prepared retinal capillaries from 6-12 diabetic and control mice by trypsin digestion⁸ and examined them by light and scanning electron microscopy. Capillaries from mice that were diabetic for 6 months revealed a moderate decrease (~50%) in the number of pericytes with the loss of the usual

Fig. 1 Kidney sections from uninfected controls and EMC-infected mice that were diabetic for 6 months. Light microscopy of a, control mouse showing normal glomeruli, tubules and Bowman's capsule (double arrows); diabetic mouse (6 months duration) showing prominent nodular glomerulosclerosis (long arrows) and thickening of Bowman's capsule (double arrows) (periodic acid-Schiff, ×145). Transmission electron micrographs of kidney from a diabetic mouse (d) showing marked thickening of the peripheral glomerular capillary basement membrane (I-I) compared with an uninfected control (c) (×15,750). Scanning electron micrograph of kidney from diabetic mouse showing f, focal effacement of the normal glomerular epithelial cells with loss of foot processes (arrow), compared with e, the usual surface pattern of epithelial cells having abundant arborizing foot processes (double arrow) normal kidney (×3,700). Sections were prepared by standard methods and examined using a Phillips 400 electron microscope or a JEOL SM 35 scanning electron microscope.

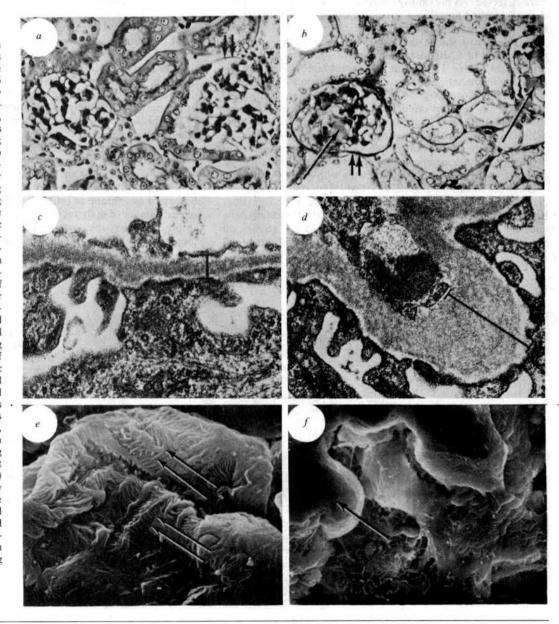


Table 1 Blood glucose levels and mortality of EMC-infected diabetic and non-diabetic mice

Days after infection	No. of animals at start of each period	Infected								
		Diabetic			Non-diabetic			Uninfected controls		
		Glucose	Deaths/ diabetics	Mortality (%)	Glucose	Deaths/ diabetics	Mortality (%)	Glucose	Deaths/ controls	Mortality (%)
7-30	100	426 ± 94	6/85	7.1	174 ± 29	0/15	0	156 ± 5	0/89	0
30-60	94	401 ± 92	16/78	20.5	166 ± 19	0/16	0	152 ± 14	1/89	1.1
60-90	79	367 ± 81	10/61	16.4	163 ± 13	0/18	0	152 ± 11	4/88	4.6
90-120	68	332 ± 77	16/43	37.2	159 ± 14	0/25	0	154 ± 12	5/85	5.9
120-150	52	306 ± 70	9/21	42.9	167 ± 20	0/31	0	159 ± 16	2/80	2.5
150-180	43	290 ± 44	7/12	58.3	168 ± 23	1/31	3.2	162 ± 19	3/78	3.9

A mouse was scored as diabetic if the non-fasting glucose level was ≥ 3 s.d. above the mean of uninfected controls. The glucose concentrations shown are the mean \pm s.d. at the beginning of each period; those shown for the infected non-diabetic animals are < 3 s.d. above the mean of uninfected mice. Fourth column, number of deaths at end of each period divided by number of diabetic mice at beginning of each period. Column 7, number of deaths at end of each period divided by number of non-diabetic mice at beginning of each period. Column 10, number of deaths at end of each period divided by number of uninfected controls at beginning of each period.

1:1 ratio of pericytes to endothelial cells (Fig. 2c,d). In $\sim 60\%$ of the diabetic mice, the retinal capillaries showed a minimal to moderate (twofold) increase in the thickness of the basement membranes. Microaneurysms were not detected in any of the animals by the PAS-trypsin digestion method^{8,9} and no cataracts were detected. As previously observed¹⁰, the pancreatic tissues showed marked atrophy of β cells in the islets of Langerhans and preservation of acinar tissue.

Thus we have shown that the decrease in the mean blood glucose level of EMC-infected mice over the 6 months of the experiment (Table 1) was due to several factors. First, many of the highly diabetic mice died, leaving survivors having much lower blood glucose levels. Second, monthly monitoring of individual animals revealed that the blood glucose of these animals also decreased with time (data not shown). Moreover, $\sim 20\%$ of the originally diabetic animals converted to the non-diabetic state by 150 days after infection. Whether regeneration of β cells or other compensatory factors is responsible for the decrease in the severity of the diabetes with time is unknown.

Using the M variant of EMC virus, which produces less severe diabetes, and by examining mice 4 months after infection, Kanich et al.¹¹ observed some mesangial thickening in diabetic mice by transmission electron microscopy. In our studies on the D variant of EMC virus, not only did we find mesangial

thickening, but also an increase in the thickness of the peripheral glomerular basement membrane and Bowman's capsule. In addition, we found prominent nodular and diffuse glomerul-sclerosis, as well as afferent and efferent arteriosclerosis. These changes are compatible with the renal complications seen in human diabetes mellitus, especially of the Kimmelstiel-Wilson type¹². The most striking similarity to human diabetic nephropathy was the change in the peripheral glomerular basement membrane, which was thickened by as much as fourfold compared with control mice. Such changes have not been well documented in alloxan-induced or streptozotocin-induced diabetes, which generally have been associated with varying degrees of mesangial thickening without significant thickening of the peripheral gomerular basement membranes^{9,13}.

The histopathological changes seen in the cornea of our diabetic mice were not dissimilar to those which predispose to corneal erosion in human diabetes¹⁴. Moreover, the decrease in the number of pericytes in the retinal vessels of diabetic animals, with the loss of the usual 1:1 ratio of pericytes to endothelial cells and the moderate increase in thickness of the basement membrane of retinal capillaries, is also characteristic of early stages of diabetes in man¹⁵. These changes are thought to precede microaneurysms. Microaneurysms usually occur in long-term diabetes in humans and in diabetic animals such as

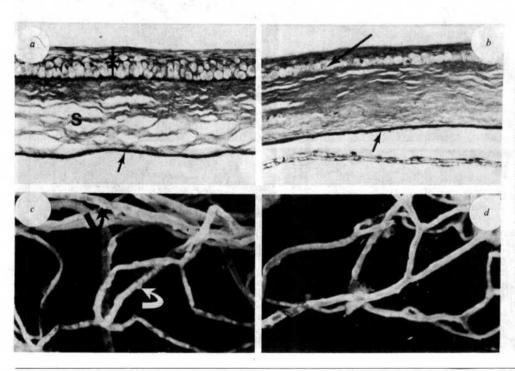


Fig. 2 The corneal epithelium of uninfected mice (a) show the usual stratification (cross) and glycogen content (periodic acid-Schiff; ×236). Cornea from mice that were diabetic for 6 months (b) show irregular stratification and moderate oedema of the epithelium, particularly of the basal layer (long arrow) (periodic acid-Schiff; ×230). The stroma (S) and Descemet's membrane (short arrow) are normal. c, Scanning electron micrograph of retinal capillaries obtained by trypsin digestion from uninfected mice, showing the normal pericyte distribution (arrows). d, A moderate decrease in the number of pericytes is observed in the trypsin digest of retinal capillaries from mice that were diabetic for 6 months $(\times 308).$

the monkey and dog, but have not been demonstrated convincingly in small animals such as the mouse and rat8. The possibility that microaneurysms may develop in EMC-infected mice that are diabetic for >6 months is now being investigated.

In conclusion, our studies in mice show that infection with the D variant of EMC virus produces both the acute and at least some of the long-term complications of IDDM.

Received 26 October 1981; accepted 12 February 1982

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Neural regulation of muscarinic receptors in chick expansor secundariorum muscle

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We have recently observed the loss of muscarinic receptors from a smooth muscle of the chicken wing, the expansor secundariorum, during post-hatch development¹. The nerve supplying the muscle has been shown to consist mainly of sympathetic fibres that, when stimulated, cause muscle contraction by the release of noradrenaline and subsequent activation of α adrenoreceptors. Although no acetylcholine release has been detected in isolated nerve-muscle preparations2, the presence of a cholinergic input has not been clearly excluded. Kuromi and Hagihara2 observed that atropine was ineffective in blocking the nerve stimulation-induced contraction of the muscle, whereas phentolamine partly and guanethedine totally abolished the contraction. We have now examined the effect of denervation of the muscle 1 day after hatch on the binding of ³H-quinuclidinyl benzilate (³H-QNB), a radioligand for mus-carinic receptors^{3,4}. Our results indicate that the normal loss of muscarinic receptors from the expansor secundariorum is prevented by denervation. This observation is important as it demonstrates the regulation of a receptor population by a nerve supply that apparently does not release the corresponding transmitter.

Muscles from 1-day old birds bound 0.165±0.024 fmol of ³H-QNB per mg wet weight. In 3-week old chicks that were unilaterally denervated at 1 day old, no significant loss of binding sites occurred (0.140 ± 0.018 fmol per mg wet weight, P>0.1), whereas binding in the contralateral, innervated muscle was significantly lower (0.093 ± 0.013, fmol per mg wet weight, P < 0.001). An anomalous, small difference in the binding was detected between the contralateral, innervated muscle and muscle from normal, unoperated birds (Fig. 1).

The weight of this muscle increased after denervation⁵, the average weight of the denervated muscles being 15.8 ± 1.5 mg. which was 48% greater than for innervated muscles (10.7± 1.0 mg; $P \ll 0.001$, n = 32). Consequently, the difference in ³H-QNB binding between the innervated and denervated muscles was exaggerated when expressed as amount bound per whole muscle. The total ³H-QNB bound by the denervated muscles was 1.98 ± 0.15 fmol per muscle and this was 2.1 times the amount bound by the innervated muscle (0.90±0.08 fmol per muscle).

The effectiveness of sympathetic denervation procedures was verified in several animals by fluorescence histochemistry. Stretch preparations of glyoxylic acid-treated muscle showed a dense innervation of the normal muscle but almost complete absence of fibres in the denervated tissue. Occasionally a few regenerating fibres were observed at the edge of the muscle.

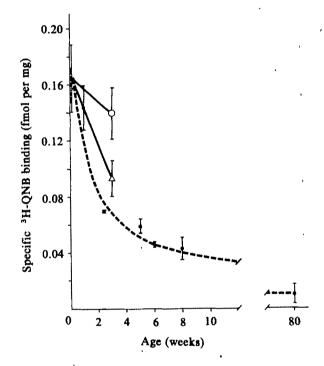


Fig. 1 Effect of denervation on the muscarinic receptor population of the developing chicken secundariorum muscle. The muscarinic receptor population of unoperated chicks at various ages (broken lines) is compared with that of chicks denervated unilaterally at 1 day old and killed 3 weeks later (solid lines). The open circles indicate the ³H-QNB bound by denervated muscles, and the open triangles the contralateral, control muscles. Denervation of the muscle was performed unilaterally by cauterization of the nerve 1 cm from its entry into the muscle. Three weeks after denervation, muscles were excised and homogenized with a glassglass hand homogenizer (Wheaton Scientific) in 1.5 ml of 50 mM phosphate buffer, pH 7.4. After centrifugation at 18,000g for 4 min, pellets were resuspended in 5 or 10 volumes of buffer. Triplicate 200-µl samples were incubated at 37 °C for 1 h with ³H-QNB (1 nM, NEN) in a total volume of 240 µl. Incubation was terminated with the addition of 1 ml of buffer and centrifugation for 4 min at 18,000g. Following two buffer washings, pellets were resuspended in 200 µl of distilled water, added to 15 ml Triton X-100/toluene scintillation cocktail and counted in a Searle Nucleonics β -spectrometer at an efficiency of ~40%. As 10-20 mg wet weight of original muscle were required for each determination, it proved necessary to pool varying numbers of muscles. Each point represents a minimum of 11 individual determinations, and is expressed as the mean ± s.e.m. All statistical values were obtained using a two-tailed Student's t-test.

Several radioligands have been used to estimate muscarinic binding sites in brain⁷, sympathetic ganglia⁸ and smooth muscle^{3,4}. These studies have indicated that the use of radiolabelled muscarinic antagonists and in particular 3H-quinuclidinyl benzylate results in binding to a single site that is accepted as the muscarinic receptor. Our previous studies with the expansor secundariorum have shown that the binding characteristics of the radioligand in this tissue are similar to those observed in other tissues, saturation occurring at 0.6 nM and with a K_D of 0.089 nM¹. We have therefore used the binding of ³H-QNB to muscle membrane as a measure of the muscarinic receptor population.

Trophic regulation of skeletal muscle function has long been the subject of intensive investigation^{9,10}. In contrast, few studies have examined the trophic effect of autonomic innervation on smooth muscle function. Indeed, until recently there has been little evidence that postganglionic sympathetic fibres exert a trophic influence on their effector cells. Unlike skeletal muscle, smooth muscle demonstrates a remarkable degree of autonomy. In particular, atrophy does not occur following denervation but functional integrity is retained.

Our results provide strong evidence for a trophic regulation of smooth muscle function by sympathetic nerves. Prevention of the normal loss of ³H-QNB binding sites from the developing expansor secundariorum by denervation suggests that their production is normally repressed by a mechanism of neuronal origin that is likely to be mediated by transmitter molecules, specific trophic molecules or electrical activity within the muscle. Whether the low level of muscarinic receptors in this tissue has any function in the young animal has not been

The composition of the nerve supplying the expansor secundariorum has not been fully established. Although it is clear that there is a dense adrenergic innervation of the muscle¹ information has been presented concerning a sensory input. Nerve stimulation has been shown to cause muscle contraction via noradrenaline release, with no evidence of acetylcholine release². These studies clearly show that the nerve regulates the muscarinic receptor population, but do not demonstrate whether this regulation is mediated by noradrenergic fibres, sensory fibres or other as yet unidentified nerve types. The evidence against the involvement of cholinergic nerves is not conclusive, but makes it unlikely that acetylcholine is involved. Histochemical and biochemical analyses over the entire developmental period are necessary to exclude this possibility. Further studies examining the origin of the nerve supply to this muscle would also aid in assessing the factor or factors involved in the regulation of the muscarinic receptor population.

Finally, the presence of a discrete nerve supply and tendon to the expansor secundariorum, together with the results of the present study, suggests that this unique muscle will be a useful tissue in which to study the interaction between smooth muscle and its innervation.

This work was funded by the NH and MRC of Australia.

Received 9 October 1981; accepted 15 February 1982

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Chronic continuous administration of neuroleptic drugs alters cerebral dopamine receptors and increases spontaneous dopaminergic action in the striatum

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The 'dopamine hypothesis' of schizophrenia states that the illness is due to overactivity of dopamine mechanisms in the brain. This hypothesis is based on two facts: (1) drugs, such as amphetamine, that enhance dopaminergic neurotransmission in the brain, may occasionally provoke a schizophrenic psychosis; and (2) acute administration of neuroleptic drugs, which are used to treat schizophrenia and other psychotic illnesses, causes blockade of brain dopamine receptors and initiates a chain of compensatory events which attempt to overcome such an action. We have previously shown that administration of neuroleptic drugs to rats for up to 18 months produces unexpected effects^{1,2}: after 6 months, all signs of blockade of dopamine receptors in the striatum have disappeared, and thereafter striatal dopamine receptors increase in number and become behaviourally supersensitive to administered dopamine agonists such as apomorphine. We now show that such chronic exposure to neuroleptics completely alters other characteristics of striatal dopamine receptors, and that in the intact animal, these changes are associated with spontaneous overactivity of striatal dopaminergic mechanisms. Unless other dopaminergic brain areas, for example in the mesolimbic or mesocortical regions, react differently from the striatum, the mode of action of neuroleptics, and the dopamine hypothesis of schizophrenia, may have to be reconsidered.

We first investigated the changes in the biochemical characteristics of striatal dopamine receptors which take place during 12 months' continuous administration of the neuroleptic trifluoperazine dihydrochloride to rats in two separate experiments-in one of them the drug was subsequently withdrawn and the animals were followed for a further 6 months (Fig. 1). The characteristics of striatal dopamine receptors were examined in these animals by estimating ³H-spiperone (0.125-4.0 nM) binding to striatal homogenates, the specificity of binding to dopamine receptors being defined by incorporation of dopamine $(10^{-4} \,\mathrm{M})$. After the first 3 months of trifluoperazine treatment there was a progressive increase in the number of striatal dopamine receptor binding sites (B_{max}) for ³H-spiperone binding in the drug-treated animals, compared with the agematched controls. B_{max} continued to increase until administration of the drug ceased after 1 yr; the number of striatal dopamine receptors then began to revert towards control levels after 3 months' drug withdrawal, and had reached control values after 6 months' drug withdrawal.

Receptor affinity, as judged by the dissociation constant (K_d) for ³H-spiperone binding to striatal tissue homogenates, also changed during 1 yr administration of trifluoperazine. In the first 3 months, K_d increased, as predicted because of the presence of drug in the tissue analysed, but after 6 months of drug administration K_d had completely or partially reverted to control levels. Thereafter, in both experiments, K_d progressively

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rose again, until the drug was withdrawn, when it abruptly reverted to control values. These changes could be explained if the drug levels had fallen, despite continued administration, perhaps due to enzyme induction, but this would not explain why K_d rose again between 6 and 12 months of trifluoperazine administration. In any case, measurement of the plasma concentration of the drug showed a gradual increase throughout this period of administration (G. Watkins, R. Whelpton and S. Curry, personal communication). The change in striatal dopamine receptor affinity after 6 months of trifluoperazine intake occurred simultaneously with the increase of dopamine receptor numbers (B_{max}) , suggesting that a new population of dopamine receptors of higher affinity than normal might have appeared; but a Hill plot of the data for 3H-spiperone binding to striatal homogenates did not deviate from unity. Whatever the reason for the change in the characteristics of striatal dopamine receptors after 6 months of trifluoperazine administration, and we can offer no explanation, the change

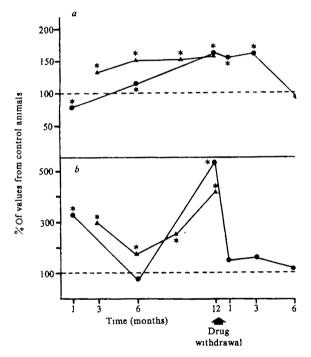


Fig. 1 Comparison of the alterations in dopamine (10⁻⁴ M)-specific ³Hspiperone (0.125-4.0 nM) binding to rat striatal preparations produced by the administration of trifluoperazine dihydrochloride continuously for up to 12 months in two distinct studies a, Receptor numbers (B_{max}) ; b, dissociation constant (Kd). The experimental groups were as follows. Expt 1: ● male Wistar rats (initial weight 200±12g; Olac International) received trifluoperazine dihydrochloride (2.5-3.5 mg per kg per day, Smith, Kline and French) for up to 12 months when the drug was withdrawn. Expt 2: A: male Wistar rats (initial weight 176±4 g, Bantin and Kingman) received trifluoperazine dihydrochloride (4.5-5 6 mg per kg per day) for up to 12 months. In each case a group of age-matched control animals from the same supplier received distilled water alone and was maintained alongside the drug-treated animals. At intervals during the course of the experiments, striatal preparations from drug-treated and control animals were examined for dopamine (10⁻⁴ M)-specific ³H-spiperone (26 Cl , Amersham) binding, according to the method of Leysen et al. Rats from age-matched control and drug-treated groups (n = 7) were killed by cervical dislocation and decapitation and the brain rapidly removed and placed on ice. The paired corpora striata were removed and placed in ice-cold 50 mM Tris buffer pH 7.7. Specific binding of 0 125-4.0 nM ³Hspiperone, as defined in the presence and absence of 10-4 M dopamine, was determined in triplicate on the pooled striatal tissue from both groups The data obtained were subjected to Scatchard analysis to determine the number of binding sites, expressed as pmol per g wet weight of tissue (K_d) , and the dissociation constant (K_d) . Statistical analysis of the receptor binding data was carried out using linear regression analysis of the results for drug-treated and control animals, which were compared using a twotailed Student's t-test *P<0.05. For comparison of the dissociation constant (K_d) and receptor numbers (B_{max}) in different animal groups at different time intervals, values in drug-treated animals are expressed as a percentage of K_d and B_{max} values in tissue from control animals obtained on the same day and assayed in parallel throughout the experiment.

Table 1 The effect of continuous administration for 14 months of distilled drinking water containing cis-flupenthixol (0 8-1.2 mg per kg per day), compared with distilled drinking water alone, on apomorphine-induced stereotyped behaviour and striatal acetylcholine concentrations in rats

Drug treatment (mg per kg per day)	Stereotypy score	Striatal acetylcholine (µg g ⁻¹)
Controls (distilled	2 ± 0 (6)	4.1 ± 0.3 (8)
water alone) cis-flupenthixol (0.8-1.2)	$3.9 \pm 0.1 * (6)$	$7.4 \pm 0.7^*$ (8)

Male Wistar rats (unitial weight 200 ± 12 g, Olac International) received cisflupenthixol hydrochloride (0 8–1.2 mg per kg per day; Lundbeck) for 14 months. A group of age-matched controls receiving distilled water alone was maintained alongside the drug-treated animals. Apomorphine-induced (0 5 mg per kg subcutaneously 15 min previously; Macfarlan Smith) stereotypy was assessed according to the scale: 0=normal; 1=continuous locomotor activity, discontinuous sniffing; 2=continuous sniffing; 3=discontinuous biting, licking and gnawing, 4 = continuous biting, licking and gnawing¹² The results are expressed as the mean (±1 s e.m.) for six animals in each group. Acetylcholine levels were determined by bioassay The caudate muclei of treated and control rats were rapidly dissected from the brain and homogenized in 1% acetic ethanol (1 ml glacial acetic acid in 99 ml absolute ethanol) and stored at -70 °C until required. Each sample was removed from the deep freeze and rotary evaporated to dryness 4 ml of frog Ringer were added to the extract and the pH was adjusted to 3.0 where necessary. The extract was then assayed on the eseminzed frog rectus using the method of Chang and Gaddum¹³, more recently described by Hebb and Silver14. Samples of the extract were compared with standard solutions of acetylcholine. A quantity of boiled alkali-treated sample equal to that of the unknown was added with each standard 15 to destroy the acetylcholine, and the addition of this as a control ensured that any substances (such as potassium) in the sample capable of causing a contraction of the rectus were not assayed as acetylcholine. To sensitize and stabilize the muscle preparation, 0.5 mg of Na-ATP was added with each sample and standard to the muscle bath. The assay used in this way was accurate to within 10%. Values in parentheses give the number of

observations.

* P < 0.05 compared with control animals, a two-tailed Student's t-test was used for parametric data; Mann-Whitney U-test was used for non-parametric results.

coincided with the onset of a behaviourally supersensitive response by the rats to the administration of dopamine agonists.

In the second series of experiments rats received cis-flupenthixol hydrochloride continuously for periods of up to 18 months. These animals demonstrated the same changes in B_{max} and K_d for specific binding of ³H-spiperone to striatal preparations. K_d initially increased, then reverted towards control levels at around 6 months, only to increase again by 12-18 months of drug intake (data not shown). At 18 months, prolonged neuroleptic intake had not only caused an increase in the number of striatal dopamine receptors, but also altered the relative sensitivity of the animals to the behavioural effects of dopamine agonists and antagonists. Whereas administration of apomorphine hydrochloride (0.125-1.0 mg per kg subcutaneously) to animals receiving such neuroleptic treatment resulted in an enhanced stereotyped response compared with age-matched controls, administration of an acute dose of trifluoperazine dihydrochloride (1-8 mg per kg intraperitoneally) produced less catalepsy in cis-flupenthixol-treated animals than in the control group (Fig. 2). Thus after 18 months' intake of cis-flupenthixol, the animals appeared to possess an increased number of striatal dopamine receptors that were supersensitive to the behavioural effects of dopamine agonists, but subsensitive to the effects of dopamine antagonists.

These results indicate that chronic neuroleptic administration alters the characteristics of striatal dopamine receptors as measured *in vitro* by receptor binding techniques, or as challenged by dopamine agonists and antagonists in behavioural pharmacological experiments. The crucial issue, however, is whether such changes are functionally significant in the intact animal or human patient.

To test this possibility, we have studied the effect of chronic neuroleptic intake on striatal acetylcholine activity. The post-synaptic striatal dopamine receptor lies, at least in part, on the cell body of cholinergic neurones, on which dopamine exerts inhibitory control^{3,4}. Alteration of striatal dopaminergic function affects acetylcholine activity. The acute administration of

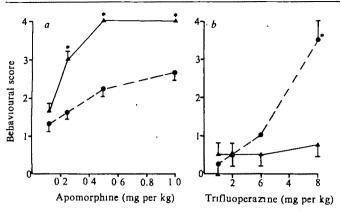


Fig. 2 Dose-response curves for a, the induction of apomorphine-induced stereotypy, and b, trifluoperazine-induced catalepsy in rats $(-\Delta -)$ receiving cls-flupenthixol (0.8-1.2 mg per kg per day) for 18 months, compared with control animals receiving distilled water alone $(--\Phi -)$. Male Wistar rats (initial weight 200 ± 12 g; Olac International) received cis-flupenthixol hydrochloride (0.8-1.2 mg per kg per day; Lundbeck) for 18 months. A group of age-matched control animals receiving distilled water alone was maintained alongside the drug-treated animals. Stereotypy induced by apomorphine (0.125-1.0 mg per kg subcutaneously 15 min previously; Macfarlan Smith) was assessed as described in Table 1 legend. The results are expressed as the mean (±1 s.e.m.) for six animals in each group. Catalepsy induced by trifluoperazine dihydrochloride (1-8 mg per kg intraperatoneally 3 h previously; SKF) was assessed as the time a rat maintained its front paws over a metal bar 2 cm in diameter and 7 cm from bench level and was scored according to the scale: 0-9 s = 0; 10 s-2.5 min = 1; 2.6-5 min = 2; 5.1-10 min = 3, 10.1-20 min = 4; >20 min = 5. The results are expressed as the mean (± 1 s e.m.) for six animals in each group. *P < 0.05 compared with control animals using a Mann-Whitney U-test for non-parametric data

neuroleptics decreases the content of acetylcholine in the striatum, due to an increased use of acetylcholine in active cholinergic neurones⁵⁻¹¹. If chronic neuroleptic administration causes a functional reversal of initial dopamine receptor blockade in the intact animal, the striatal acetylcholine content should increase, due to a decrease in cholinergic activity resulting from enhanced dopaminergic inhibition of striatal cholinergic neurones.

We produced striatal dopamine receptor supersensitivity in rats by the continuous administration of cis-flupenthixol for 14 months and examined changes in striatal acetylcholine content in the same animals. At the end of 14 months treatment with cis-flupenthixol, the rats exhibited an enhanced behavioural response to the dopamine agonist apomorphine, and basal striatal acetylcholine content had doubled (Table 1). This result suggests that after chronic cis-flupenthixol treatment, not only was the response of striatal dopamine receptors to an exogenous dopamine agonist no longer antagonized, but also the receptors were overactive in the intact animal. In other words, the chronically neuroleptic-treated rat striatum exhibited spontaneous dopaminergic synaptic overactivity.

In conclusion, chronic neuroleptic intake by rats for periods of up to 12-18 months changes fundamentally the character of striatal dopamine receptors, which increase in number, but also exhibit unexplained alterations in their affinity. Such changes are associated with parallel alterations in the response of the animals to exogeneous dopamine agonists and antagonists. In addition, and most important, there are also changes in striatal acetylcholine function which suggest that the spontaneous activity of at least some striatal dopamine receptors is increased in the chronically neuroleptic-treated animal. These events may be responsible for the emergence of tardive dyskinesias in man, and they must be taken into account when considering the mechanism of action of neuroleptics and the pharmacological basis of schizophrenia.

This study was supported by the Wellcome Trust, MRC and the Research Funds of the Bethlem Royal and Maudsley Hospital and King's College Hospital. We thank Smith, Kline and French and Lundbeck for generous drug supplies.

Received 16 July 1981, accepted 15 February 1982

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Specific non-opiate binding sites for human β -endorphin on the terminal complex of human complement

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The opioid peptide β -endorphin is released from the pituitary into the blood under physical or emotional stress; its target tissues, however, are unknown¹. We report here the specific binding of human β -endorphin to both terminal complexes of human complement: the cytolytic, membrane-derived C5b-9(m) complex and the cytolytically inactive, serum-derived SC5b-9 complex. Binding is through the carboxy-terminus of B-endorphin rather than the amino-terminus, which is implicated in binding to opiate receptors. It is conceivable that the binding of β -endorphin to complement has physiological relevance in the adaptation of the immune system to stress

Human serum or plasma drawn in 10 mM EDTA was obtained from healthy donors. The complement pathway was activated by adding 2 g crushed inulin (Merck) to 100 ml serum followed by incubation at 37 °C for 4 h. Activation by inulin leads to the formation of the macromolecular, non-cytolytic SC5b-9 complex (comprising of complement components C5b, C6, C7, C8, C9 and the S-protein²). Supernatants obtained after centrifugation of the inulin-serum suspension were used directly in binding experiments or served as a starting material for the isolation of the SC5b-9 complex^{2,3}. The terminal, cytolytic complement complex, C5b-9(m), which consists of complement components C5b to C9 without the S-protein, was generated on target sheep erythrocyte membranes by lysis of the antibody-coated cells with human serum, and then isolated from detergent solubilizates of the membranes as described previously⁴. Detergent-solubilized, hypotonically lysed sheep

erythrocyte membranes served as controls.

125I-labelled β -endorphin was obtained by iodinating human B-endorphin (Bachem) using published techniques^{5,6}. Binding of human β -endorphin to the terminal C5b-9 complexes was demonstrated in a radioreceptor assay and by sedimentation analysis in sucrose density gradients.

In the radioreceptor assay, a small amount of human ¹²⁵I-\(\beta\)endorphin was found to bind to one or several components of native human serum (Fig. 1). After inulin activation, however, binding increased considerably (Fig. 1).

When ¹²⁵I-β-endorphin was incubated with native serum or with EDTA-plasma and the samples were then centrifuged through linear sucrose density gradients, total radioactivity was recovered in the top fractions of the gradient (not shown). In a parallel experiment using inulin-activated serum, however, ¹²⁵I-β-endorphin was also recovered in the fractions (22–23S) known to contain the SC5b-9 complement complex³.

The identity of SC5b-9 with a β -endorphin-binding component in inulin-activated serum was confirmed by the demonstration that purified SC5b-9 exhibited β -endorphin-binding capacity in the radioreceptor assay (Fig. 1). ¹²³I- β -endorphin also co-sedimented with isolated SC5b-9 in sucrose density gradients (not shown). Further experiments showed that human ¹²⁵I- β -endorphin was almost completely displaced from its binding sites on the SC5b-9 complement complex by unlabelled human β -endorphin (Fig. 1).

Saturable binding sites for human β -endorphin were also found on the SC5b-8 and SC5b-7 complexes of human complement, but not on the isolated C3 or C9 components (L.S., S.B.

and H.T., unpublished observations).

 125 I- β -endorphin also bound to the isolated, cytolytic C5b- 9 (m) complement complex, whereas no binding was observed to hypotonically lysed sheep erythrocyte membranes (radio-receptor assay data are shown in Fig. 1). Consistent with this finding, total radioactivity was recovered from the top fractions of density gradients when β -endorphin binding to control mem-

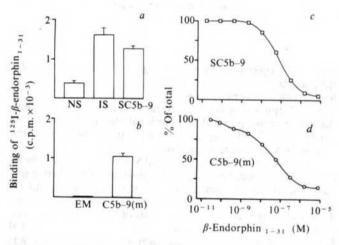


Fig. 1 a, b, Binding of human $^{125}\text{I-}\beta$ -endorphin₁₋₃₁ (c.p.m.) to native human serum (NS); inulin-activated serum (IS); the cytolytically inactive, terminal complex of human complement (SC5b-9); control erythrocyte membranes (EM); and the terminal, cytolytic complex of human complement [C5b-9(m)]. Mean values \pm s.d. from three determinations. c, d, Binding of human 125 I- β -endorphin₁₋₃₁ to the terminal SC5b-9 or C5b-9(m) complexes of human complement in the presence of various concentrations of unlabelled human β -endorphin₁₋₃₁ (binding expressed as per cent of total binding, specific plus nonspecific, as determined in the absence of β -endorphin₁₋₃₁; 100% corresponds to the binding values for SC5b-9 or C5b-9(m), respectively, shown to the left). Binding was determined by radioreceptor assay (a modified radioim-munoassay technique^{5,6}): 150 µl buffer (0.02 M sodium phosphate, 0.15 M NaCl, 0.01% bovine serum albumin (BSA), 0.1% gelatin, 0.01% thiomerosal, pH 7.5) were incubated with 50 µl serum or 50 µl complement complex or membrane solution (adjusted to 300 or 30 µg protein per ml buffer for displacement or saturation binding experiments respectively) and with 20 μ l buffer containing 125 I- β -endorphin and 0.1% Triton X-100. In addition, 20 µl buffer containing 0.1% Triton X-100 were added; in the competition experiments, the buffer also contained unlabelled inhibitor. Total concentration of 125 I- β -endorphin in radioreceptor assays was adjusted to ~8,000 c.p.m. per tube corresponding to 0.35 nM. The samples were incubated at 4 °C for 18 h. Then 300 µl of ice-cold charcoal suspension (1 g charcoal and 0.5 g BSA in 100 ml buffer) were added to adsorb free labelled or unlabelled ligands. After 10 min incubation at 4 °C the samples were centrifuged and the radioactivity in the supernatants was measured as an indication of the amount of ¹²⁵I-β-endorphin bound to complement complex.

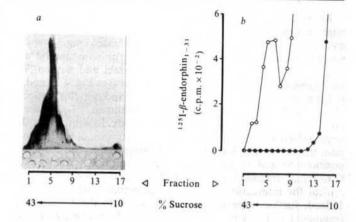


Fig. 2 Binding of human ¹²⁵I-β-endorphin₁₋₃₁ to C5b-9(m) demonstrated by co-sedimentation in a sucrose density gradient. a, Fused rocket immunoelectrophoresis²² (using a specific antiserum to identify C5b-9(m) complex) from fractions of density gradient centrifugation of a sample containing C5b-9(m) complex. b, Radioactivity determined in the fractions from a density gradient centrifugation of samples containing $^{125}I-\beta$ -endorphin incubated with either C5b-9(m) complex (O) or detergent-solubilized control erythrocyte membranes (). No radioactivity sedimented in the control experiment. In the experiment performed with C5b-9(m) complex, however, the radioactivity peak coincided with the peak of immunoprecipitate; no protein was found in the upper fractions (9-13) of the gradient, indicating that the radioactivity in these fractions reflects ^{125}I - β -endorphin dissociated during centrifugation. For density gradient centrifugation 50 µl 12 I-B-endorphin solution (4 nM) were incubated at 22 °C for 20 min with either 250 µl inulin-activated serum or SC5b-9 or C5b-9(m) complex, or, as controls, with saline, EDTA-plasma or erythrocyte membranes. Samples were then centrifuged at 8 °C through 12.4 ml linear 10-43% (w/v) sucrose density gradients (containing 25 mM Tris buffer, 50 mM NaCl, 5 mM NaN3, 2.4 mM Triton X-100, 2.5 mM DOC pH 8.2) at 35,000 r.p.m. in a SW41 Ti rotor (Beckman L2 65 B) for 16 h (ref. 3). Fractions were collected from the bottom of the tube and assayed for radioactivity.

branes was tested (Fig. 2); however, on centrifugation of the C5b–9(m) complex incubated with 125 I- β -endorphin, radioactivity was also found in the fractions (25–40S) which contained the complex⁷ (Fig. 2). Figure 1 shows displacement of 125 I- β -endorphin from the C5b–9(m) complement complex by unlabelled β -endorphin.

To test the reversibility of the binding reaction, we performed chase experiments. Samples containing C5b–9(m) were incubated with ^{125}I - β -endorphin for 18 h at 4 °C. Subsequent addition of unlabelled β -endorphin resulted in a decrease of ^{125}I - β -endorphin binding providing evidence for the reversibility of ^{125}I - β -endorphin binding to the C5b–9(m) complex. The shapes of the displacement curves (Fig. 1d; Fig. 4) and a Scatchard analysis of binding data (Fig. 3b) suggested negative cooperativity or several classes of binding sites. Assuming two classes of binding sites, we calculated two corresponding $K_{\rm d}$ values from the Scatchard plot (Fig. 3b) of about 80 and 3 nM respectively; the latter was in reasonable agreement with a $K_{\rm d}$ of about 1 nM estimated from the binding curve of a saturation experiment (Fig. 3a).

Classical opiate receptors are known to bind the N-terminal fragment of β -endorphin: β -endorphin is displaced from these binding sites by opioid peptides such as Met- and Leuenkephalin, and by the opiate antagonist naloxone. None of these substances, in concentrations up to $10 \,\mu\text{M}$, was able to inhibit $^{125}\text{I}-\beta$ -endorphin binding to the C5b-9(m) complex. Thus the N-terminal fragment of β -endorphin is obviously not essential for the binding of human β -endorphin to the human complement complex C5b-9(m).

To determine which fragment of human 125 I-B-endorphin might interact with the C5b-9(m) complex, competition experiments were performed using several unlabelled fragments of human β -endorphin and camel β -endorphin as inhibitors (Fig. 4). The amino acid sequences of camel and human β endorphin differ only at positions 27 and 31 (see Fig. 4 legend). β -Endorphin₁₋₁₆, human β -endorphin₁₋₂₇ and camel β -endorphin₁₋₃₁ all failed entirely to compete with human ¹²⁵I-β-endorphin₁₋₃₁ in binding to the C5b-9(m) complex. In contrast, both human β -endorphin₁₋₃₁ and human β -lipotropin₁₋₉₁ which contains the amino acid sequence of human β -endorphin between positions 61 and 91, displaced $^{125}I-\beta$ -endorphin₁₋₃₁. Thus, the C-terminal fragment of human β -endorphin seemed to be critical for interaction between β -endorphin and C5b-9(m). Indeed, further experiments performed with the C-terminal fragment Lys-Gly-Glu of human β -endorphin showed that this tetrapeptide was capable of displacing ¹²⁵I- β -endorphin₁₋₃₁ from the C5b-9(m) complex (Fig. 4); however, the concentrations required were about 1,000-fold higher than those of human β -endorphin₁₋₃₁. All the data therefore support the existence of novel, non-opiate binding sites on the C5b-9(m) complex for the C-terminal fragment of β -endorphin. The relatively high concentrations of the C-terminal tetrapeptide required to displace ¹²⁵I-\(\beta\)-endorphin suggest that other regions of the peptide may also contribute to the binding.

The structural specificity of β -endorphin binding was further confirmed by the demonstration that $^{125}I-\beta$ -endorphin was not displaced from the C5b-9(m) complex by other peptides (10 μM) such as adrenocorticotropin₁₋₂₄, insulin, luteinizing hormone-releasing factor, dynorphin₁₋₁₃ or β -casomorphin₁₋₇. Human leukocyte interferon⁸ and IgG⁹ have been reported to interact with endorphin antisera; however, neither human fibroblast interferon nor IgG and its Fab or Fc fragments affected the binding of ¹²⁵I-\(\beta\)-endorphin to the C5b-9(m) complex.

The binding of human β -endorphin to the terminal C5b-9(m) complement complex thus exhibits several characteristics of a ligand-receptor interaction: reversibility, structural selectivity, saturability and high affinity; and it is tempting to assume that the process is biologically significant. Indeed recent results are consistent with an involvement of endorphins in the immune system: a transport system for benzomorphans in leukocytes has been reported¹⁰; opioids apparently influence plasma levels of interferon¹¹; α -endorphin-immunoreactive material has been detected in plasma cells¹²; and there is evidence for the

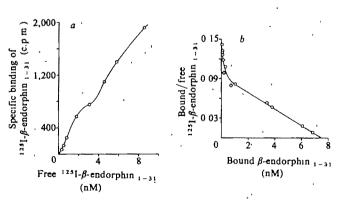


Fig. 3 a, Saturation experiment: Increasing concentrations of human 125 1- β -endorphin₁₋₃₁ were incubated with C5b-9(m) (6 μ g protein per ml) in the presence or absence of 1.7 μ M human β -endorphin₁₋₃₁. Free ¹²⁵I- β -endorphin was plotted against specifically bound (displaceable by 1.7 μ M unlabelled β -endorphin) ¹²⁵I- β -endorphin. Mean values from four determinations (s.d. ± <4%). Biphasic curves were obtained in several experiments. b, Scatchard analysis of binding data obtained by displacement²³ of human ¹²⁵I- β -endorphin by unlabelled human β -endorphin (Fig. 4). Specifically bound/free ¹²⁵I- β -endorphin is plotted as a function of total (labelled plus unlabelled) ligand specifically bound.

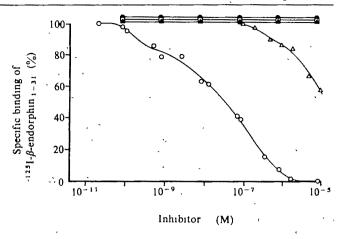


Fig. 4 Specific binding of human ¹²⁵I-β-endorphin₁₋₃₁ to C5b-9(m) in the presence of various concentrations of unlabelled binding inhibitors: \square , β -endorphin₁₋₁₆; \blacktriangle , human β -endorphin₁₋₂₇; \bullet , camel β -endorphin₁₋₃₁ (C-terminal amino acid sequence His-Lys-Lys-Gly-Gln); O, human β-endorphin₁₋₃₁ (C-terminal amino acid sequence Tyr-Lys-Lys-Gly-Glu); and Δ , human β -endorphin₂₈₋₃₁ (amino acid sequence Lys-Lys-Gly-Glu). Mean values from three determinations (s.d. \pm <4%). Specific binding represents bound ¹²⁵I- β -endorphin, that is displaced by 8.3 μ M unlabelled human β -endorphin₁₋₃₁.

presence of opioid receptors on phagocytic leukocytes¹³ and T lymphocytes^{14,15} as well as for non-opiate β -endorphin receptors on cultured lymphocytes¹⁶.

 β -Endorphin appears to be released from the pituitary into the blood under physical or emotional stress¹⁷⁻¹⁹ and during parturition^{20,21}. This might represent an adaptation of the immune system to stress situations.

We thank Margit Roth, Mechthild Vogel and Silvia Wilhelm for technical assistance, Professor Glossmann for valuable discussions, Dr Li for human β-lipotropin₁₋₉₁ samples and Dr Hofstetter for the gift of IgG and its Fab and Fc fragments. This study contains parts of the thesis of L. Schweigerer to be presented to the Fachbereich Humanmedizin in partial fulfilment of a doctorate of medical science. The work was supported by Deutsche Forschungsgemeinschaft (SP Neuroendokrinologie and grant We 340/10).

Received 28 September 1981, accepted 17 February 1982

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Parental exposure to X rays and chemicals induces heritable tumours and anomalies in mice

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Although radiation and various chemicals are known to produce mutations, there is a paucity of information on the production of germ-line mutations leading to heritable tumours and anomalies. I now report a significant and large increase in such tumours and anomalies following treatment of ICR parent mice with X rays and urethane. About 90% of the induced tumours were in the lung, and were inherited as if they were dominant mutations with about 40% penetrance.

X-ray irradiation was chosen because of the substantial previous experimental work on radiation mutagenesis ¹⁻⁶. Urethane is known to be mutagenic, carcinogenic and teratogenic in mice and has also been used widely as a human drug. Additional experiments, with similar results, were done with 4-nitroquinoline 1-oxide (4-NQO). The present report summarizes the data from large-scale experiments involving 12,905 live-born mice and 9,645 fetuses from 2,904 parents studied during 1967–81 (the spontaneous and induced rates of tumours and anomalies have not changed noticeably in this period). The experimental procedures and detailed results will be published elsewhere.

The first comparison between radiation and urethane was in the production of dominant lethals. As shown in Fig. 1, there was a significant, almost linear increase in dominant lethals following irradiation of spermatids and spermatozoa. Similarly, there was an increase of dominant lethals following treatment of mature oocytes, with no significant fractionation effect (although the data, taken at face value, suggest a slight reduction with fractionation). On the other hand, treatment of spermatogonia produced no significant increase, although the numbers are too small for a small change to be detected. These results are in accord with previous studies8-11. In contrast, examination of 5,830 embryos revealed no significant increase of dominant lethals from urethane treatment (1.5, 2.0 and 2.25 mg per g) at any stage. Nevertheless, as will be shown below, urethane is very effective in producing tumours and anomalies.

As shown in Table 1, there is a large and significant increase in anomalies in the progeny of parents treated with radiation or urethane. Generally, a higher rate of anomalies is detected prenatally than after birth, because many of the anomalies (for example, cleft palate, exencephalus, gastroschisis and buphthal-

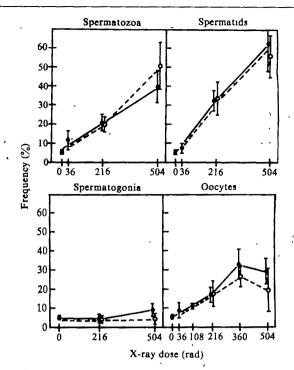


Fig. 1 Per cent of dominant lethals in the progeny of mice irradiated at the stages and dosages shown • Acute doses, O, fractionated doses, given in 36 rad amounts at 2-h intervals. Dominant lethals were measured by the mean number of early deaths per implant Vertical bars indicate the 90% confidence limits of the mean computed from the t distribution. For spermatozoa treatment males were mated 1-7 days after irradiation, for spermatid 15-21 days, and for spermatogonia 64-80 days; for mature occytes at the late follicular stage, females were irradiated 1-14 days before ovulation.

mus) are lethal shortly after birth. Only open eyelid, the predominant type among non-lethal anomalies, was shown to be transmissible (see Table 1 legend).

Figure 2 gives the dose-response data for radiation and the effects of dose-fractionation. The data are too limited to permit any conclusions about the shape of the dose-response relationship for male treatment, although there is a clear increase with dose in females. As with dominant lethals, the treatment of spermatogonia was less effective than post-meiotic treatment. There is again a suggestion of a fractionation effect for oocyte treatment.

Both radiation and urethane significantly increase the number of tumours in the progeny, regardless of which sex is treated (Table 1). The dose-response data for radiation (Fig. 3) show a clear increase with dose for treatment of post-meiotic stages in males and less clear results for spermatogonial treatment. Occytes at late follicular stages were resistant to 36-108 rad of X rays, but very sensitive to higher doses. Both the

Table 1	Incidence of	tumours and	i anomalies	in the offs	pring of mic	ce exposed	to X rays o	r urethane

•	Treatment to pa	Dose	Anomalies de 19-day-old		Anomalies de 7-day-old o		Tumours in o	
Agent	Sex	(rad or mg per g)	Incidence	(%)	Incidence	(%)	Incidence	(%)
X rays	М	36-504	48/2,201	(2.2*)	16/2,396	(0.7)	153/1,529	(10 Ot)
X rays	F	36504	25/942	(2.7*)	29/1,750	(1.7†)	101/1,155	(8 7‡)
Urethane	M	2.0	10/477	(2.1t)				
Urethane	. · · M	1.5	65/2,923	(2.2*)	16/1,637	(1.0)	136/1,254	(10.9†)
Urethane	F	1. 5	52/1,262	(4.1*)	22/1,087	(2.0*)	139/963	(14.4*)
Urethane	F	1.0		, ,	12/885	(1.4†)	115/772	(14.9*)
Untreated		00	4/1,026	(0.4)	1/809	(0.1)	29/548	(5.3)

[&]quot;X-ray irradiation was by Toshiba KC-18-2A, 180 KVp at a rate of 72 rad min⁻¹. Data are totals for various dosages, fractionations and age of exposure. Urethane was given as a single subcutaneous dose. Pathology was detected both by gross examination and histologically. The induced anomalies were cleft palate, kinky and/or short tall, dwarf, open eyelid, exencephalus, hydrocephalus, gastroschisis, polydactyly, syndactyly, gigantic toe, buphthalmus, hydatid mole, atresia hymenalis, mislobulation of lung or liver, and hemiplegia. Among non-lethal anomalies, open eyelid, the predominant type (41%), was transmissible to f_2 , but transmission of tail anomaly was inconsistent. Other non-lethal anomalies are sterile or too rare to examine exact heritability. Types of induced tumours are given in the text. Results were significantly different from the control value at $^*P < 0.001$, $^*P < 0.001$ and $^*P < 0.005$ by χ^2 .

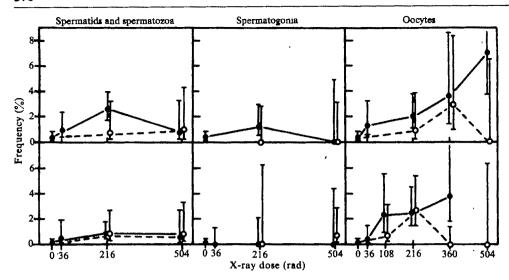
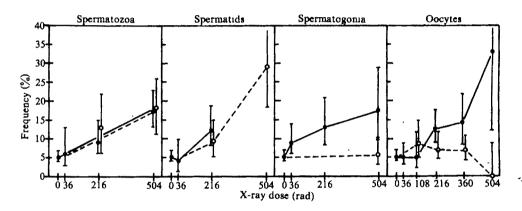


Fig. 2 Frequency of prenatal (above) and postnatal anomalies (below) in the progeny of parents irradiated in a single dose (●) and fractionated (○). Frequency of the anomaly was given by per cent of anomalous progeny in survivors. Vertical bars indicate 90% binomial confidence limits. For treatments, see Fig. 1 legend. Although data are not shown, spermatocytes at 22–42 days before mating were twice as sensitive to X rays as were post-meiotic sperm. Further study on dose-response relationship remains with these stages.

Fig. 3 Frequency of tumours in the progeny of mice irradiated at various stages and doses. • Acute doses; O, fractionated doses. Tumour frequency was given by per cent of tumour-bearing progeny in survivors. Vertical bars indicate 90% binomial confidence limits. For treatments, see Fig. 1 legend.



spermatogonial and oocyte treatment effects of dose-fractionation suggest some repair at high doses. Eighty-seven per cent of the induced tumours were in the lung (papillary adenomas), the remainder being of various types—ovarian tumour, lymphocytic leukaemia, stomach tumour, lipoma, granulosa cell tumour, thyroid tumour, liver haemangioma and hepatoma. The spectrum of tumour incidence in the offspring of treated animals is essentially the same as in controls (90% in the lung).

To confirm that the induced tumours are heritable, the F_1 progeny of treated parents were mated and their progeny examined. In addition to those tumours induced by urethane and radiation, some induced by 4-NQO were included. The mice were mated as young adults and the presence or absence of tumours was determined at 8 months of age. The progeny were then classified as to the retrospectively determined type of the parent. The data are shown in Table 2. In each case the original treated mouse was a male, as a precaution against the possible transmission of the chemical to the progeny. The results were confirmed by continuing the urethane-treated group into the F_3 generation. The F_3 results were: $+\times-$, 5/22; $-\times-$,

Table 2 Lung tumours in F2 progeny of treated parents

Phenotypes	Parental treatment		
of F ₁ parents	X rays	4-NQO	Urethane
+×- -×-	15/76 (19 7*) 4/69 (5 8)	7/28 (25 0†) 5/103 (4.9)	4/19 (21.2) 9/113 (8.2)

Male parents were treated with X rays (504 rad), 4-NQO (10 μ g per g) or urethane (1.5 mg per g) at 8-14 days before mating. + Means that the F₁ mouse later developed lung tumour, - means that it had not developed a tumour by 8 months. There was significant difference between +×- and -×- mating at *P<0.05 and †P<0.01.

5/125 (see Table 2 legend for explanation of symbols). Combining the data, the proportion of affected progeny from $+\times-$ matings was 21.4%. The pattern of inheritance is that of a dominant with about 40% penetrance. Reduced penetrance was also found for dominant skeletal mutations⁶.

In addition, four untreated females that later developed spontaneous lung tumours produced 49 progeny. Of these, only two (4.1%) developed tumours, a value not different from the progeny of normal mice in this strain. The histological pattern was the same in the two groups. One possible explanation for the different heritability is that the spontaneous tumours were caused by somatic rather than germinal mutation. In view of the quantitative doubts about spontaneous rates, doubling dose calculations are not presented.

Although tumours were produced by both radiation and urethane, urethane failed to produce a significant increase of dominant lethals, suggesting that gross chromosomal rearrangements are not produced by urethane. To test this further, progeny of treated parents were examined for translocations detected cytologically at meiosis. Of 64 progeny from parents treated with 504 rad of X rays at post-meiotic stages, 10 had characteristic translocation configurations. Two others had small testes with no meioses, probably also caused by gross chromosomal rearrangements. One of these two had a lung tumour. Among the 54 progeny mice showing no evidence of translocations, eight had lung tumours. In contrast, no evidence of translocations was found in 50 progeny of parents treated with urethane. This difference between X rays and urethane was confirmed by genetic experiments with Drosophila melanogaster⁷. Urethane produced no chromosome rearrangements or dominant lethals, but was a potent mutagen. At the highest doses, the number of recessive X-linked lethals was equivalent to those produced by 2,000 rad of y rays.

The fact that urethane produces tumours but not translocations or dominant lethals and the observation that tumours occur no more frequently in those mice with X-ray-induced translocations (1/12) than in those without translocations (8/54) argues that the mutations producing tumours are not related to gross chromosome changes. There could, of course, be cytological changes (small deletions, for example) not detected as translocations. In any case, the tumours behave as dominant mendelian factors with a penetrance of about 40%. Reduced penetrance is not surprising in this kind of trait.

It is striking that tumour mutations occur about 10 times as frequently as dominant skeletal mutations^{4,5}. The spontaneous tumour incidence is also much higher (~5%) than skeletal mutations. It is possible that many more gene loci are involved¹²; alternatively, specific loci may be especially prone to lung tumour-causing mutations in my ICR strain.

The most important reservation regarding these experiments

Received 10 November 1981, accepted 26 January 1982

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is that the results may be a special property of the mouse strain used. However, other kinds of tumours (ovarian tumours and lymphocytic leukaemias), although much less frequent than lung tumours, increased in the same ratio. It is important that similar experiments should be done with other mouse strains to test the generality of the results. Confirmation of the findings in other strains would mean that heritable cancer induced by radiation and chemical mutagens becomes an important factor for study in human populations such as those of Hiroshima and Nagasaki. Note that in traditional experiments designed to test mutability in mice the progeny are not kept long enough for tumours to develop.

I thank Dr J. F. Crow for advice and help in summarizing this work, the late Dr J. Kamahora, and Drs Y. Sakamoto, S. Kondo and E. Okamoto for their encouragement, and Drs A. G. Searle and P. C. Hoppe for their critical comments. The work was supported by JMESC grant 56480373.

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A host gene controlling early anaemia or polycythaemia induced by Friend erythroleukaemia virus

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Friend ervthroleukaemia virus (FLV) is a type-C murine retrovirus that induces a rapid multi-stage erythroleukaemia in susceptible mice¹⁻⁵. An early phase (1-3 weeks) is characterized by splenomegaly and rapid changes in erythropoiesis⁵⁻⁸, while a later stage (1-3 months) is associated with the development of clones of highly tumorigenic cells^{2-5,9}, which can be established into Friend erythroleukaemic cell lines. Two distinct isolates of FLVs, both of which can induce the early and late stages of the diseases, have been described. The original isolate (FV-A) induces a rapid severe anaemia, while a later isolate (FV-P)¹⁰, derived from stocks of FV-A, induces a rapid polycythaemia7. In addition to the contrasting changes in haematocrit, the diseases induced by FV-A and FV-P can also be distinguished. For example, while the erythropolesis in FV-A infected mice appears to remain under the control of the hormone erythropoietin^{8,11,12}, erythropoiesis in FV-P infected mice is erythropoietin-independent^{8,11-13}. Furthermore, Friend erythroleukaemic cell lines derived as a consequence of FV-A and FV-P infection also display different cellular properties^{5,9,14}. Here we demonstrate that in addition to the viral contributions, host genes play an important part in determining the nature of the disease induced by FV-A or FV-P. A new host gene which determines the induction of early anaemia or polycythaemia by FLV is described.

The polycythaemia strain of FV-P has been used extensively in studies of host genetic control as well as its interaction with the haematopoietic progenitor cells^{15,16}. Most of such studies used mice strains DBA/2 and BALB/c. We have now extended these studies to other mice strains, notably CBA. Figure 1a illustrates the change in haematocrit in DBA/2 mice following the infection by FV-A and FV-P. The data show that for FV-A, the haematocrit of the mice dropped from a normal value of 50% to ~35% by 2-3 weeks, while that of FV-P infected mice increased to over 70% during the same period. However, as also shown in Fig. 1a, following the infection of FV-P into another strain of mouse CBA, instead of increasing, the haematocrit fell to ~35%. A similar reduction of the proportion of red cells in the circulating blood was also detected following infection with FV-A. This implies that not only can a contrasting response in haematocrit values be stimulated by FV-A and FV-P, but that different haematocrit values can be induced after infection of FV-P into two different strains of mice. These rapid changes in haematocrit appear to be the effect of the replication-defective spleen focus-forming virus components, SFFV_A and SFFV_P (ref. 8) of FV-A and FV-P, respectively. The helper virus, F-MuLV, did not induce such changes in either DBA/2 or CBA mice (Fig. 1a).

It is possible that CBA mice are resistant to polycythaemia induction by FV-P and that the early anaemia observed in these mice after injection of FV-P is due to a small quantity of contaminating SFFVA in the FV-P stocks. To rule out this possibility, we have prepared a FV-P preparation by transfection of moleculary cloned polycythaemia strain of spleen focus forming virus (SFFV_P) DNA¹⁷ to NIH/3T3 cells and rescued these transfected cells with cloned F-MuLV¹⁷. Data in Fig. 1b indicate that this virus preparation, which contained only virus from molecularly cloned SFFV_P and F-MuLV, also induced an early polycythaemia (70% haematocrit) in DBA/2 and a rapid anaemia (30% haematocrit) in CBA mice. Another possibility is that inoculation of FV-P into CBA mice resulted in certain host-induced changes in the viral genomes, or that endogenous FV-A was induced, thus causing anaemia in these mice. To rule this out, a 10% spleen extract was obtained from the CBA mice 2 weeks after infection with FV-P and injected into five DBA/2 and five CBA mice. Results indicate that, similar to the original stock of FV-P, the virus in these spleen extracts induced a severe polycythaemia (average haematocrit 69± 2.5%) in DBA/2 mice and a rapid anaemia (haematocrit 40± 4%) in CBA mice 2 weeks after inoculation.

We next examined several inbred mouse strains and their changes in haematocrit after infection by FV-P. Mouse strains DBA/2, CBA, C3H, AKR and BALB/c were injected with an equal dose of FV-P and their haematocrits measured at the time of infection (0 week) and 2 weeks later. Results in Table 1 indicate that, like that of DBA/2 mice, the AKR mice and BALB/c mice strains developed rapid polycythaemia. The C3H strain, however, responded like CBA mice in developing an anaemia. These contrasting results demonstrate that host determinants have an important role in determining these early erythropoietic changes.

To examine if such contrasting effects in the changes of haematocrit as a result of infection by FV-P is the result of a single locus gene, (CBA×DBA/2) F₁ hybrid mice and mice from backcrosses of DBA/2×(CBA×DBA/2) F_1 and CBA× (CBA×DBA/2) F₁ were developed and their haematocrit changes 2 weeks after injection with FV-P were measured. The data in Fig. 2 indicate that, similar to the findings described above, all the DBA/2 mice injected with FV-P developed a rapid polycythaemia with a haematocrit value of about 65-70%, while the haematocrits of all the CBA mice dropped to 35-40%. The (CBA \times DBA/2) F_1 hybrids, however, developed a slight polycythaemia, with haematocrit values between those of CBA and DBA/2 mice, 55-60%. These data suggest that if the early anaemia or polycythaemia is controlled by a single gene, the gene has a co-dominant effect. The haematocrits of mice from $CBA \times (CBA \times DBA/2)$ F_1 appear to segregate into two groups, one group with haematocrits of 35-40% and another with values of 55-60%, while the haematocrits of the DBA/2 \times (CBA×DBA/2) F₁ mice seem to have segregated into two groups, one at 55-60% and one at 65-70%. These results indicate that the haematocrit values in the hybrid mice segregated as single genotypes, suggesting that the induction of early

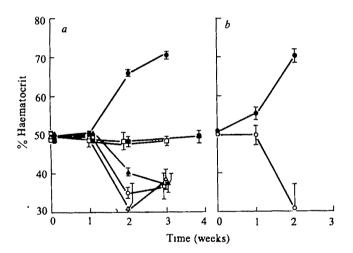


Fig. 1 a, Kinetics of changes in haematocrit values in DBA/2 and CBA mice infected with FV-P, FV-A or F-MuLV. Clonal isolates of FV-P, FV-A or F-MuLV used were described elsewhere. To examine the kinetics of changes in haematocrit values, 2×10³ focus-forming units (FFU) of FV-P, 10³ FFU of FV-A, or 2.5×10⁶ XC plaque units of F-MuLV were injected through the tail veins of female DBA/2 mice or CBA female mice (Jackson Laboratories). At weekly intervals, the haematocrit values of the mice were determined by bleeding through the tail veins of the mice with haematocrit tubes (Dade Division, American Hospital Supply, Miami). At least four mice were used for each determination. All mice were 7-10 weeks old at the time of infection. Symbols: ●, FV-P into DBA/2; O, CBA; ♠, FV-A into DBA/2 and CBA, △; ■, □ F-MuLV into DBA/2 or CBA. Bar represents 1 s.e. b, Changes of haematocrit values in DBA/2 and CBA mice after infection of viruses from molecularly cloned SFFV_p DNA and F-MuLV. Molecularly cloned polycythaemia strain of spleen focus-forming virus (SFFV_p) DNA were transfected to NIH/3T3 cells¹⁷. The transfected cells were rescued with cloned F-MuLV^{8,17}, the virus in the supernatant was injected into either DBA/2 or CBA mice and their haematocrit values were determined as described for a. Symbols: •, DBA/2; O, CBA mice. At least four mice were used in each determination. Bar represents 1 s.e.

Table 1 Early changes in haematocrit in different mouse strains following infection by FV-P

, , , , , , , , , , , , , , , , , , ,	Наета	tocrit
Mice	0 week	2 weeks
DBA/2	51.0 ± 1.0	66.5 ± 0.9
BALB/c	52.0 ± 1.5	57.3 ± 3.1
AKR	49.3 ± 0.9	71.0 ± 2.4
CBA	49.5 ± 0.5	36.8 ± 3.7
СЗН	49.8 ± 0.5	40.0 ± 1.9

2×10³ FFU of FV-P were injected into DBA/2, BALB/c, AKR, CBA or C3H mice and the haematocrit values were determined at the time of infection and 2 weeks later (see Fig. 1). All mice used were female, 7-10 weeks old at the time of infection. At least four mice were used in each determination. Error represents 1 s.d.

polycythaemia or anaemia in DBA/2 and CBA mice, respectively, is controlled by a single co-dominant locus.

The contributions of host hereditary factors in determining the susceptibility to FLV infection is well established¹⁴⁻¹⁶. Several host genes—Fv-1 (ref. 15), Fv-2 (refs 16, 18, 19), W (refs 8, 20), Steel (refs 8, 16, 21, 22) and H-2 (refs 16, 23)—are known to control the susceptibility of mice to erythroleukaemia induction by this virus. The present gene, however, seems to affect the type of erythropoietic modulations by FLV rather than the susceptibility.

This finding, taken together with the previous reports that the types of modification of the erythropoiesis patterns depend on the strain of FLV used^{8,11,12}, indicate that the modulation of the haematopoietic organization by FLV is the result of a complex array of interactions between host genes and the viral genomes. These early experiments showed that, following infection by FV-A and FV-P, certain pathological differences could 🎺 be detected. For example, FV-P induces a rapid polycythaemia associated with erythropoietin-independent poiesis^{8,11-13}, while FV-A induces a rapid anaemia associated with eporythropoietin-dependent erythropoiesis^{8,11,12}. The reason for these contrasting changes is not known. Perhaps the two viruses infect different target cells in the haematopoietic hierarchy, leading to the modulation and appearance of cells with different properties. This hypothesis is also supported by the nature of the tumorigenic colonies emerging in the late stages of the disease^{5,9}. These colonies, which are probably the result of progressions from the infected cells in the early phase of the disease, when derived from FV-P- or FV-A-infected mice, differ in their kinetics of appearance⁵, their self-renewing capacities, their incidences of chromosomal aberrations and their responsiveness to induction by erythropoietin^{9,14}

The present data indicate that in addition to the contributions of host genes in determining the susceptibility to infection by FLV, the outcome of the virus-induced erythropoietic modulations is under genetic control. We propose that this locus in DBA/2 and CBA mice, which controls the state of erythropoiesis early after infection by FV-P, be designated Fv-5. Thus, CBA mice which develop early anaemia after infection with FV-P have a genotype of $Fv-5^{aa}$ and DBA/2 mice which develop polycythaemia, Fv-5^{pp}. The mode of action of this host gene is unknown. Although it is possible that the viruses infect different target cells in CBA and DBA/2 mice, leading to the infection or transformation of non-erythroid cells or of erythroid cells with different differentiation potentials, preliminary results do not support this. Similar to DBA/2 mice infected with FV-P, the CBA spleen cells are predominantly erythroblasts. Furthermore, erythropoiesis of the FV-P-infected CBA A mice and the formation of erythroid colony-forming units were also independent of any added erythropoietin (T.S., Y. Niho and T.W.M., unpublished data). Alternatively, this gene may affect the severity of haemolysis of the FLV-transformed erythroid cells, or control the rate of proliferation and differentiation of late erythroid cells. Finally, it may function by controlling the transport of the transformed erythroid cells from the bone

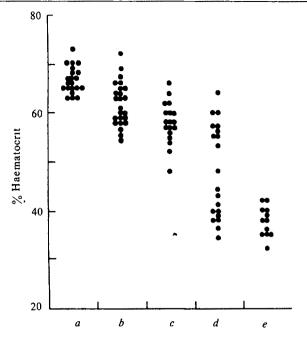


Fig. 2 Haematocrit values of: a, DBA/2; b, (CBA×DBA/2)F₁; c, (CBA×DBA/2)F₁; d, CBA×(CBA×DBA/2)F₁; e, CBA mice weeks after infection by FV-P. 2×10³ FFU of FV-P were injected into each mouse with background of DBA/2, CBA, $(CBA \times DBA/2)F_1$, $DBA/2 \times (CBA \times DBA/2)F_1$ and $CBA \times DBA/2$ (CBA×DBA/2)F₁, and their haematocrit values were determined 2 weeks after infection. (CBA×DBA/2) F_1 , DBA× $(CBA \times DBA/2)F_1$ and $CBA \times (CBA \times DBA/2)F_1$ mice were developed in the animal colony of the Ontario Cancer Institute.

marrow or spleens of these mice, thus controlling the proportion of red cells in the circulation. Comparison of the properties of the erythroid progenitor cells, the rate of erythropoiesis and the nature of tumorigenic Friend erythroleukaemic cell lines derived after infection of mice of genotypes Fv-5^{aa}, Fv-5^{ap} and Fv-5^{pp} will help to elucidate the process of leukaemic transformation by FLV and cellular heterogeneity of leukaemic clones.

This work was supported by the MRC of Canada. Note added in proof: Recent results from this laboratory suggest that Fv-5 controls the rate of proliferation of a late (post CFU-E) erythroid precursor cell(s).

Received 12 August 1981, accepted 16 February 1982

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Unequal crossing-over accounts for the organization of *Drosophila* virilis rDNA insertions and the integrity of flanking 28S gene

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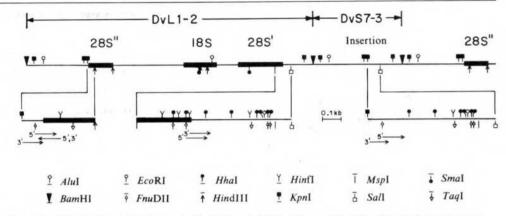
The majority of ribosomal DNA (rDNA) repeats in Drosophila melanogaster and Drosophila virilis have an insertion in the 28S rRNA coding region, and such repeats are essentially inactive in transcription1. There are two unrelated types of insertion in D. melanogaster rDNA, one of which (type 1) has some homology with insertions in D. virilis2, and is located in precisely the same position as that in the D. virilis rDNA³⁻⁶. D. melanogaster type 1 rDNA insertions fall into three size classes of 5, 1, 0.5 kilobases (kb), and sequences of shorter insertions comprise the downstream end of longer ones^{4,5} Indeed, the points at which the sequences of longer type 1 insertions depart from shorter ones are oligonucleotides that are closely related to a 14-base pair (bp) rRNA coding sequence directly repeated at the flanks of D. $virilis^3$ and some D. melanogaster4 rDNA insertions. It has been suggested that shorter D. melanogaster type 1 insertions derived from longer ones by unequal crossing-over that involved these homologies, and I show here that long D. virilis rDNA insertions may have arisen from shorter ones in the same way. Also, I suggest that such unequal crossing-over can generate intact rDNA repeats from those with insertions.

The major D. virilis rDNA insertion has been described as being ~10 kb long^{2,3,7}, although it had been noted that it appears to be a tandem duplication². Blot hybridizations using uncloned D. virilis rDNA digested with restriction enzyme combinations not previously used have now shown that 5-kb insertions are at least as frequent as 10-kb insertions, and that a HindIII cleavage site was misinterpreted in a previous study². Restriction site mapping and the sequencing of homologous regions of a 10-kb insertion verified that it is a tandem duplication. This fact, together with sequence data on the middle of a 10-kb insertion, strongly suggest that it arose from 5-kb insertions by unequal crossing-over, with the flanking 14-bp repeats of coding sequence serving as points of homology. Longer segments containing the insertion have been found from blots to have lengths that are also multiples of \sim 5 kb. If these are from rDNA, they presumably arose from unequal crossing-over between rDNA repeats having insertions of various lengths. However, they may represent non-rDNA copies of the insertion 1,2,6

The D. virilis rDNA clones pDvL1-2 and pDvS7-3 used in this study have been described elsewhere^{2,3,8}; the map of an rDNA repeat that contains a 10-kb insertion is shown in Fig. 1, together with the lengths of rDNA that were sequenced for this study.

Figure 2 (lane 2) shows the hybridization of DvS7-3, a BamHI segment of D. virilis rDNA that contains only insertion sequences (Fig. 1), to D. virilis nuclear DNA digested with SmaI+ HindIII and electrophoresed in a 0.4% agarose gel. There are no SmaI or HindIII sites in the known D. virilis rDNA insertions, but there is a SmaI site in the 28S' coding region ~2.2 kb upstream of the insertion (Fig. 1), and a HindIII site 280 bp downstream of the insertion in the 285" coding region3. The greatest amount of hybridization occurred to a band at 7.5 kb, and this length is attributable to a 5-kb insertion plus flanking coding sequences to the SmaI and HindIII sites. The next most intense band is at ~12.8 kb, and is considered to derive from rDNA repeats having 10-kb insertions. Other bands, indicated

Fig. 1 Maps of the cloned D. virilis rDNA segments DvL1-2 and DvS7-3 that together represent an rDNA repeat having a 10-kb insertion in the 28S rRNA coding region². DvL1-2 itself may be a segment derived from two adjacent repeats with 5-kb insertions, or from adjacent repeats with insertions of different size, but the contiguity in rDNA of BamHI segments comparable with DvL1-1 and DvS7-3 has been demonstrated². The distribution of restriction enzyme cleavage sites in the insertion shown indicates that it is a tandem duplication, and a



comparison of DNA sequences around the BamHI, EcoRI and SalI sites in DvL1-2 and DvS7-3 has verified this. The horizontal arrows beneath the expanded portions of the map indicate DNA that was sequenced specifically for this study. Sequencing was done according to Maxam and Gilbert¹³. DNA segments were either 5' end-labelled with $[\gamma^{-32}P]ATP$ and polynucleotide kinase¹³, 3' end-labelled with $[\alpha^{-32}P]dNTP$ and the Klenow fragment of DNA polymerase¹⁴, or 3' end-labelled with $[\alpha^{-32}P]3'dATP$ and terminal transferase¹⁵.

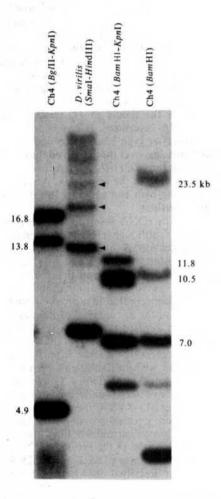


Fig. 2 Hybridization of the rDNA insertion segment DvS7-3 (Fig. 1) to a blot of *D. virilis* nuclear DNA digested with Sam → Sma and HindIII and electrophoresed in a 0.4% agarose gel. Conditions have been described elsewhere⁸. With respect to insertions in the 28S coding region, the nearest SmaI site is ~2.2 kb upstream in the 28S' portion, and the nearest HindIII site is 278 bp into the 28S" portion³. Lanes flanking the D. virilis lane contain markers of end-labelled segments of Charon 4 DNA ^{5,16}: the first lane shows a KnpI digest of Charon 4 (Ch4) end-labelled at BgIII sites; the third lane shows a KnpI digest of Charon 4 end-labelled at BamHI sites; fourth lane, BamHI segments of Charon 4. The sizes of some segments are indicated in kilobases¹⁶. Before blotting, DNA in the gel was fragmented by depurination to facilitate transfer of high-molecular weight DNA to the nitrocellulose filter¹⁷. Arrowheads indicate bands that are ~5-kb multiples of the darkest band at 7.5 kb.

by arrowheads in Fig. 2, are also 5-kb multiples of the 7.5-kb band, and the ladder may extend beyond the measurable range of segment sizes.

I determined the sequence of the very centre of a duplication to determine the precision of a tandem array. Figure 3 (centre) shows the sequence within the 0.7-kb *KpnI/SalI* segment of DvS7-3 that spans the end of one 5-kb insertion and the beginning of the next (Fig. 1). Also shown for comparison are the termini of insertions in the clone DvL1-2 (ref. 3). These are exactly represented in DvS7-3. Furthermore, they are separated in DvS7-3 by a 14-bp sequence, TGTCCCTATCTACT, that flanks insertions as a direct repeat, and is present as a single copy in an uninterrupted unit of rDNA^{3,5}.

The data indicate that tandem repeats of a 5-kb rDNA insertion arose from unequal crossing-over in which the 14-bp flanking sequence served as the point of sequence homology. An event generating a 10-kb insertion from such recombination between two repeats having 5-kb insertions is shown in Fig. 4.

The reciprocal of the product with a 10-kb insertion is an rDNA repeat having no insertion (Fig. 4), and presumably recombination between intact genes and interrupted ones can also have occurred (this is the reverse of the event shown in Fig. 4). Thus it is possible that there is a flux between populations of rDNA repeats having 28S coding-region insertions and rDNA repeats that are intact. This has a bearing on an enigmatic feature of Drosophila rDNA: while repeats with insertions are inactive in rRNA synthesis9, and the insertions themselves are probably ancient and are greatly divergent among Drosophila species2, coding-region sequences that flank insertions are largely indistinguishable from sequences in intact, functional genes^{3,5}. This is unexpected for gene copies that appear to have been nonfunctional for a very long time, particularly when insertion sequences immediately adjacent to coding regions are evidently under little or no pressure for sequence conservation^{2,5}. However, if coding regions in interrupted genes can be dissociated from insertions through unequal crossing-over involving the duplicated coding sequence at the flanks of insertions, and can exchange sequences with functional genes, then sequence integrity in the coding regions of interrupted genes would have been maintained.

There is one known case in which coding sequences adjacent to an insertion are not intact, that of the 5-kb type 1 insertion in *D. melanogaster* rDNA. There is a 23-bp deletion of coding sequence at the 28S' end of such insertions^{5,6}, and this deletion includes the 14 bp that are directly repeated at the ends of other insertions. Associated with the deletion are some other defects in the 28S' portion of the coding region⁵. One rDNA clone of three that have been examined in detail has another upstream deletion of 49 bp, and the results of blot hybridizations involving unfractionated rDNA have indicated the existence of other aberrations in the 28S' region of genes with

(285' coding region)

DVL1-2 TAGTGACGCGCATGAATGGATTAGGAGATTCCTACTGTCCCTATCTACTCAGTTCGTTTCAGACAGTCGTTGGGAACAGACGTGT 3-DVS7-3 CGCGGCAGGTGCTCACGTTAAGGCCCACTGACTTTCATGTCCCTATCTACTCAGTTCGTTTCAGACAGTCGTTGGGAACAGACGTGT 3

Dvl1-2 CGCGGCAGGTGCTCACGTTAAGCCCACTGACTTTCATGTCCCTATCTACTACTACCGACACCAGCCAAGGGAACGGGCTTGGA 3 (285° coding region)

Fig. 3 A comparison of sequences at the ends and at the centre of a 10-kb rDNA insertion in D. virilis. The middle line is from a KpnI/SalI segment of DvS7-3 (Fig. 1) that covers the junction of tandem repeats in a 10-kb insertion. The top line shows the sequence containing the junction between the 28S' rRNA coding region and one insertion in DvL1-2, and the sequence in the bottom line covers the 28S" end of the other insertion (see Fig. 1). Single underlining indicates the termini of insertions, and double underlining represents the 14 bases of 28S rDNA that are duplicated at the flanks of D. virilis rDNA insertions. Such a direct repeat of coding sequence at the flanks of insertions relates the insertions to transposable elements³. The identity of sequences in the DvS7-3 segment with those of DvL1-2 junctions strongly suggests that the 10-kb tandem duplication resulted from unequal crossing-over involving the flanks of insertions (see Fig. 4).

5-kb insertions. The 14 bp sequence is intact at the 285" end of 5-kb insertions, and no coding region defects have been associated with the 28S" coding region⁵. There are no observed tandem repeats of the 5-kb insertion as there are in D. virilis This also reflects the absence TGTCCCTATCTACT sequence at the 28S' end of 5-kb D. melanogaster insertions, as without it, unequal crossing-over events such as that shown in Fig. 4 cannot occur.

A problem with generalization of the unequal crossing-over model arises when 1- and 0.5-kb type 1 insertions in D. melanogaster rDNA are considered. These shorter insertions are flanked by identical or similar copies of the 14-bp sequence4 yet there are no observed duplications or higher multiplications of either length of insertion (only one-half of the 1-kb insertion is homologous with the 0.5-kb insertion). There is as yet no explanation for this apparent inconsistency between interrupted rDNAs in D. melanogaster and D. virilis.

Unequal crossing-over in Drosophila rDNA has been invoked to account for differences in the degree of the bobbed phenotype between certain parental strains and their progeny¹⁰ it has also been shown to occur meiotically between sister strands at the rDNA locus in yeast11. The generation of tandem multiplications of a DNA segment containing the ampC (ampicillin resistance) gene in Escherichia coli has been suggested to involve recombination between copies of a 12-bp sequence that occurs twice within 10 kb of DNA that include ampC¹². Thus there are precedents for the model given in

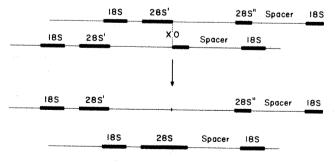


Fig. 4 Unequal crossing-over between D. virilis rDNA repeats with 5-kb insertions produces a repeat having a 10-kb insertion and one with an intact 28S rRNA coding region. The point of homology between repeats that is involved in recombination (dotted vertical line) is the 14-bp sequence directly repeated at the flanks of insertions. Crossing-over within this tetracaidecanucleotide generates a copy in the middle of the 10-kb tandem duplication, and this is indicated by a vertical dash. Sequence contexts are shown in Fig. 3.

Fig. 4. However, this is the first indication of rescue of coding sequences from an interrupted and transcriptionally inactive state to one in which the sequences can be functional in intact

I thank Bruce Kohorn, Janice Oles, Anne Scott and Robert Wade for assistance. This work was supported by NIH grant GM 26378.

Received 1 December 1981; accepted 5 February 1982.

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Flip-flop hydrogen bonding in a partially disordered system

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Cyclodextrins have proved useful as model systems for the study of hydrogen bonding¹. α -Cyclodextrin (α -CD) crystallizes from water as a hexahydrate having well defined hydrogen bonds (O-H···O) in linear and circular arrangements favouring 'endless' ... O-H... O-H... chains 1-3. The larger β -cyclodextrin (β -CD) forms a crystalline dodecahydrate, β -CD·12H2O in which X-ray localization of the hydroxyl hydrogen atoms is made difficult because 25 of the 45 hydroxyl groups are statistically disordered and water molecules enclosed within the β -CD cavity are distributed over several sites that are not fully occupied4. We have therefore carried out a neutron diffraction study of β -CD at the Oak Ridge high flux isotope reactor to determine whether there is any well defined hydrogen bonding in disordered systems. In β -CD there are 19 hydrogen bonds of the type O-H...H-O. In these bonds, oxygen atoms are in the normal O-H...O distance range⁵, but two statistically half-occupied H atoms are arranged between them. The fact that the H...H separation of ~1 A is so short that the two H atoms positions are mutually exclusive suggests an equilibrium between two states: O-H··O=O··H-O. Of the two H atoms only one is in hydrogen-bonding contact at a given time; the other one is flipped out to form a hydrogen bond with an adjacent acceptor group and vice versa. Because long hydrogenbonding chains are involved in a cooperative, concerted motion (domino effect), we have coined the term 'flip-flop hydrogen bond'. This study demonstrates that hydrogen bonds are operative in disordered systems and display dynamics even in solid state.

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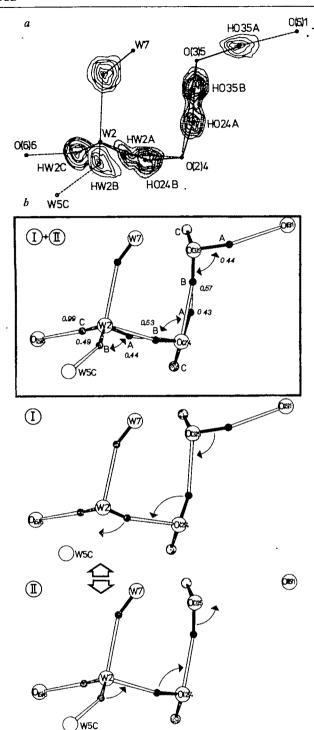


Fig. 1 a, Section of a Fourier difference map calculated from neutron diffraction data, showing D atom positions (referred to as H atoms in the text). Flip-flop hydrogen bonds Oare indicated by stippling. O, Positions of H, O and C atoms; covalent bonds; ---, hydrogen bonds. b, Same section as in a, giving an interpretation at the atomic level and showing the deconvolution of the flip-flop hydrogen bonding system into two states (I and II) having normal hydrogen bonds of the type O-H...O. Solid arrows indicate the manner in which H atoms may jump in concerted, cooperative motion from one state to another. Circles of increasing size represent H, C and O atoms, with C shown shaded and H filled. Covalent bonds are indicated by solid lines, H bonds by open lines. The water molecule, W2, displays three H atom positions, one (C) fully occupied with an occupancy factor 0.99 (1) whereas A and B are only partly filled [0.44 (1) and 0.49 (1)]. In the text, H atoms A, B and C of W2 are designated HW2A, HW2B and HW2C and those attached to hydroxyl O(2)4 are termed HO24A and HO24B. All geometrical data are close to 'standard' values and will be published in detail elsewhere.

As β -CD·12H₂O was crystallized from D₂O, replaceable hydroxyl and water H atoms were substituted by D. Here, however, we will only use the term H atom.

The hydrogen bonding scheme of β -CD was assigned from 7,378 neutron diffraction data covering a resolution of 0.56 Å. Within one asymmetric unit of space group P2₁ containing B-CD·12H₂O, there are 39 hydrogen bonds of the type O-H...O, and 19 of the type O-H...H-O in which two H atoms are located between oxygen atoms in a near-linear arrangement (Fig. 1a). In the latter, O.O separations are in the normal range (2.75-2.90 Å) as are covalent O-H distances of ~1 Å. The apparent H.··H contacts of between 0.86 and 1.18 Å, however, are much shorter than the 2.4 Å calculated from the sum of can der Waals' radii of the two H atoms6. The two H atom positions are therefore mutually exclusive and to prevent clashing, their positions are statistically only half filled and in each O-H···H-O hydrogen bond, H atom occupancies add up to ~1.0. Therefore, if one of the two H atoms is present in the O-H···H-O hydrogen bond, for geometrical reasons the other one is pushed out to form a hydrogen bond with another partner as acceptor, and vice versa. This scheme was observed in all the 19 O-H···H-O bonds. Because, on a statistical basis, the oxygen atoms in such a 'flip-flop' system are always involved in hydrogen bonding, their positions and vibrational parameters are hardly affected by changes from one hydrogen bonding direction to another. Rather, they act as 'fixed centres' around which hydrogen atoms arrange or 'flip-flop' according to optimum bonding patterns.

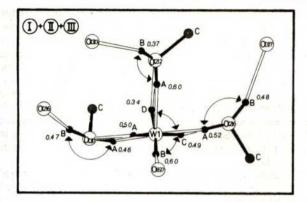
A comparable scheme has been found previously in deuterated ice^{7,8} and in ice clathrates⁹. Two deuterium atoms are located between hydrogen-bonded oxygen atoms and in ice, they are responsible for the observed residual entropy of 0.87 entropy units (ref. 10). The salient difference between ice and the system considered here, however, is that in the high-symmetry (hexagonal and cubic) space groups of ice and ice clathrate, all O and D positions are constrained by symmetry requirements, whereas they are free to arrange in β -CD·12H₂O.

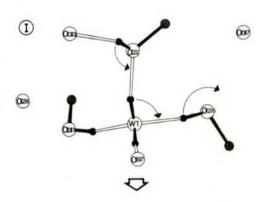
Figures 1 and 2 show examples of flip-flop hydrogen bonds. In Fig. 1, there are two flip-flop O—H···H—O hydrogen bonds connected by hydroxyl O(2)4—H, that is, W2—HW2A··· HO24B—O(2)4 and O(2)4—HO24A···HO35B—O(3)5. The H atom of O(2)4 is twofold disordered, producing HO24A and HO24B with occupation factors of 0.43 (1) and 0.53 (1). Both positions cannot be filled simultaneously. Assuming HO24A to be occupied, then the hydrogen bonding chain (I) shown in Fig. 1b is formed involving all atoms indicated by A. If, on the other hand, position HO24A is empty and HO24B occupied, then a concerted change of H atoms and hydrogen bonds occurs so that all positions indicated by B are filled (II in Fig. 1b).

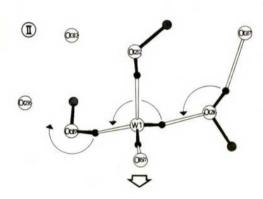
The water molecule W2 is engaged in two fully established hydrogen bonds, accepting from HW7A and donating to O(6)6, and therefore positions W2 and HW2C are fully occupied. The other hydrogen atom of W2 is part of the flip-flop system and disordered over HW2A and HW2B. All three hydrogen occupancies associated with W2 add up to 1.92 (2), in close agreement with the ideal 2.0. The two occupation factors of HW2B and HW2A, 0.44 (1) and 0.49 (1) add up to only 0.93 instead of the presumed 1.0. This is probably due to errors in measurement and refinement or is caused by incomplete H/D exchange.

In the more complicated scheme illustrated in Fig. 2, three flip-flop hydrogen bonds cluster around water molecule W1. The oxygen atom position of the latter is fully occupied, but its hydrogen atoms are statistically disordered over four tetrahedrally arranged sites with occupation factors in the range 0.34 (2)–0.60 (1), adding up to 1.93 (2). The arrangement of the water H atoms could correspond to statistical distribution over six different water orientations with unknown individual contributions.

Because the H atom positions in the flip-flop hydrogen bonds shown in Figs 1 and 2 are approximately half occupied with







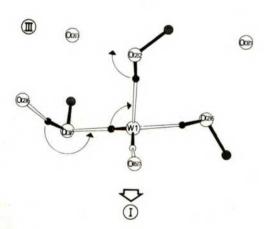


Fig. 2 A more complex system of three flip-flop hydrogen bonds connected by a water molecule, W1. The scheme observed (top) is separated into three schemes (I, II and III) having standard hydrogen bonds O-H···O; more schemes are possible because W1 is probably oriented statistically into six different positions.

occupancy factors adding up to ~1.0 in each case, the two possible hydrogen bonds O-H...O and O...H-O are energetically comparable. This means that there may be transitions from one to the other, a jump of a H atom from one position to another causing all the connected hydrogen bonds to change orientation in a cooperative, concerted mechanism, reminiscent of a relay system (domino effect; see arrows in Figs 1, 2).

The crystallographically observed atomic arrangement O-H···H-O could be due either to averaging of crystal domains or of unit cells containing hydrogen bonds O-H...O and O···H-O, or to direct observation of a dynamical equilibrium which also appears to prevail in ice9,10:

$$O-H\cdots O \neq O \therefore H-O$$

Because we do not know the residual entropy of crystalline β-CD·12H₂O, we cannot assess which of the two possibilities is more likely. In solution, however, it is certain that the dynamical system will be present. Moreover, if single 'standard' hydrogen bonds O-H...O are compared energetically with flip-flop hydrogen bonds, the latter will be preferred because of the expected advantageous entropic term resulting from statistical, near-equivalent distribution of H atoms between the oxygen atoms. In this context, it is of interest that differential scanning calorimetric measurements of β-CD·12H₂O crystals showed a strong endothermic effect at -46 °C, suggesting that below this temperature, an ordering of the whole crystal structure occurs into one or other of the flip-flop orientations11

Thus we conclude that hydrogen bonding occurs even in badly disordered systems. Note that water molecules acting as joints within a system of flip-flop hydrogen bonds click into well defined orientations dictated by optimum hydrogenbonding interactions and are by no means rotating to produce spherically averaged structures. The hydrogen bonds seem to operate in even less stringent conditions than previously assumed. This leads to flickering clusters of water occurring either in bulk or in hydration shells where disordered hydroxyl groups are dominant. If we combine flip-flop hydrogen bonds with circular structures formed by hydroxyl groups as observed in the α -cyclodextrin hydrates^{1,2,12}, an interesting dynamical system is obtained which consists of circles of four to six (or more) water hydroxyl groups with all O-H···O-H···O-H hydrogen bonds running in the same direction (homodromic, see ref. 2) and oscillating clockwise and anti-clockwise in a flip-flop manner. Such systems are favoured energetically due to the cooperative effect³ as well as to the entropic contributions of two equivalent or near-equivalent states. In water clusters, such flip-flop circles can be interconnected to form larger, rapidly interconverting and changing systems.

G.M.B. was supported by the Division of Materials Sciences, Office of Basic Energy Sciences, US Department of Energy, under contract W-7405-ENG-26 with the Union Carbide Corporation. B.H. was supported by the Office of Health and Environmental Research, US Department of Energy, under contract W-7405-Eng-26 with the Union Carbide Corporation.

Received 27 August 1981; accepted 22 February 1982.

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Conversion of the chemical energy of methylmalonyl-CoA decarboxylation into a Na⁺gradient

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Primary active transport systems establish electrochemical gradients of their transport substrates at the direct expense of chemical or light energy. The chemical energy can be provided by cleavage of the phosphoric anhydride bond of ATP, establishing Na+, K+, H+ or Ca2+ gradients with the respective ATPases. Whereas in eukaryotic cells the Na+ and K+ imbalance between cells and the surrounding fluids is brought about by the (Na++K+)ATPase1, no such enzyme is known for bacterial cells. Generally, the gradients of Na+ and K+ ions in these cells are believed to be generated by secondary transport systems consuming the energy provided by the electrochemical gradient of protons^{2,3}. In some bacterial species, however, sodium ions may be transported by primary ATP or light-driven sodium pumps^{4,5}. The primary sodium pump oxaloacetate decarboxylase represents a novel energy conversion mechanism in that it consumes the chemical energy of the decarboxylation of oxaloacetate to drive Na⁺ transport⁶⁻⁸. We show here that methylmalonyl-CoA decarboxylase is another example of a Na⁺ transport enzyme converting the chemical energy of a decarboxylation reaction into an ion gradient.

The action of oxaloacetate decarboxylase in catalysing the transport of Na⁺ ions across the cell membrane, thereby generating an electrochemical gradient of Na⁺ ions⁶⁻⁸, is in accord with its localization in the cell membrane and the specific requirement of Na⁺ ions for catalytic activity⁹⁻¹¹. By analogy, we hypothesized that methylmalonyl-CoA decarboxylase might also function in this way. As this enzyme apparently did not require a specific metal ion for catalytic activity, it could catalyse the transport of protons.

Methylmalonyl-CoA decarboxylase from *Micrococcus lactilyticus* (syn. *Veillonella alcalescens*) is reported to be tightly bound to the ribosomes^{12,13}, a property not compatible with a transport function for this enzyme. We have therefore reexamined the localization of the decarboxylase using chromatography of the particulate cell fraction on Sephacryl S-1000 Superfine columns. Ribosomes and membranes were clearly resolved, as indicated from the separation of the markers succinate dehydrogenase (for the membrane) and ribose (for ribosomes) and also from the change in the ratio of absorbances at 280 and 260 nm (~1 in the membrane and ~0.5 in the ribosomal fractions). The distribution of methylmalonyl-CoA decarboxylase indicated that it was attached to the membranes but not to ribosomes.

The enzyme was solubilized from the membranes with Triton X-100 and purified about 30-fold over the crude membrane extract on an avidin–Sepharose affinity column¹⁴. Examination of the cation requirement of the purified enzyme indicated that the decarboxylase was specifically activated by Na⁺ ions $(K_{\text{Mapp}} \sim 1 \text{ mM})$ at 0.10 mM (R, S)methylmalonyl-CoA). This property of the enzyme and its binding to the cell membrane are analogous to properties of oxaloacetate decarboxylase and are compatible with a function of the decarboxylase as a sodium pump.

Sodium transport was determined from the amount of radioactivity taken up into inverted vesicles of *V. alcalescens* on incubation with ²²NaCl. Whereas in the absence of methylmalonyl-CoA almost no Na⁺ was taken up by the vesicles, substrate addition resulted in the rapid accumulation of Na⁺ (\sim 1.1 nmol ²²Na⁺ per mg protein within the first 15 s; Fig. 1). Due to the high activity of the decarboxylase (0.35 U per mg protein), all methylmalonyl-CoA was decarboxylated within the 15 s, thus terminating Na⁺ uptake. The accumulated Na⁺ was not retained in the vesicles but returned to the medium at an initial rate of \sim 0.55 nmol per min per mg protein, probably due to leakiness of the membranes. These observations are completely analogous to the kinetics of Na⁺ transport into vesicles from *Klebsiella aerogenes* as catalysed by oxaloacetate decarboxylase⁷.

The action of methylmalonyl-CoA decarboxylase as a Na⁺ transport enzyme was confirmed by treating the vesicles with avidin, which simultaneously abolished Na⁺ transport and

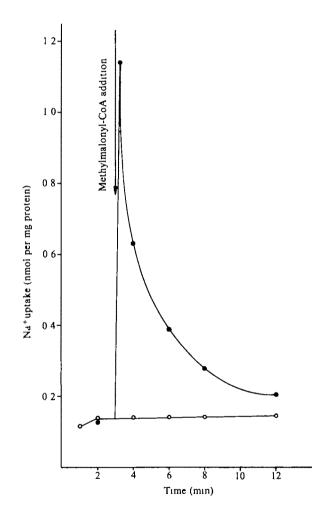


Fig. 1 Kinetics of ²²Na⁺ uptake into inverted membrane vesicles from V. alcalescens. The vesicles were prepared by passing V. alcalescens cells in 50 mM K-phosphate buffer, pH 7.5, through a French press at 8,000 p.s.i. and were subsequently isolated by centrifugation steps as described previously. The inverted vesicles (3.4 mg protein, 0.35 U per mg methylmalonyl-CoA decarboxylase) were incubated at 25 °C in a total volume of 10.67 ml 50 mM K-phosphate buffer, pH 7.5, with 0.44 mM ²²NaCl (2,800 counts per min per nmol). At the arrow, 0.3 mM (R, S)methylmalonyl-CoA (Li-Salt) was added and ²²Na⁺ uptake was determined from samples (0.1 ml) taken at appropriate incubation periods and passed over columns (0.5×2 cm) of Dowex 50, K⁺ to separate free Na⁺ from Na⁺ trapped inside the vesicles. The radioactivity of internal ²²Na⁺ was subsequently determined by liquid scintillation counting (•). O, Control without methylmalonyl-CoA addition.

Table 1 Avidin-induced inhibition of methylmalonyl-CoA decarboxylase-catalysed Na+ uptake into inverted vesicles

Pretreatment of vesicles	Methylmalonyl-CoA decarboxylase activity (U per mg protein)	²² Na ⁺ upt incubation 0.25 min (nmol per n	periods of 1 min
None Avidin-biotin	0.34	1.14	0.63
complex	0.36	1.09	0.65
Avidin None, methyl- malonyl-CoA	0	0.11	0.15
omitted	0	0.13	0.14

See Fig. 1 legend for experimental conditions.

decarboxylase activity (Table 1). A parallel incubation with an avidin-biotin complex, however, did not affect either of these activities, ruling out the possibility that the avidin might act on the transport system in any way other than by a complexing of the biotin prosthetic group of the decarboxylase. The products of the decarboxylation, propionyl-CoA and bicarbonate, were unable to substitute for methylmalonyl-CoA in inducing Na+ accumulation to any considerable extent, thus confirming that Na+ is transported during the decarboxylation reaction. The function of methylmalonyl-CoA decarboxylase as a Na+ transport enzyme was further confirmed by reconstitution experiments. When the decarboxylase was purified by affinity chromatography on avidin-Sepharose and incorporated into phospholipid vesicles by the detergent dilution method¹⁵, using octylglucoside as the detergent, the reconstituted proteoliposomes were able to concentrate Na+ ions about six times over the incubation medium in response to the decarboxylation of methylmalonyl-CoA.

The mechanism of the Na+ transport was investigated by analysing the effect of certain ionophores. If the Na+ transport was electrogenic, creating a membrane potential, this could be limiting for the amount of Na+ accumulation, as has been shown to be the case for oxaloacetate decarboxylase. The membrane potential would be discharged in the presence of valinomycin and K⁺ by the electrophoretic movement of K⁺ ions. In these conditions, about twice as much Na+ was accumulated in the vesicles as in the valinomycin-free controls (Table 2), thereby indicating the presence of electrogenic Na+ transport. The uncoupler carbonylcyanide-p-trifluoromethoxy phenylhydrazone also increased Na+ uptake within the vesicles, although less efficiently. This effect is probably also due to discharging of the membrane potential. Furthermore, the result indicates that the generation of a proton gradient is not the primary event for the Na+ transport. As expected, the accumulation of Na⁺ inside the vesicles was completely abolished by the presence of the Na⁺ ionophore nigericin.

Thus, our results characterize methylmalonyl-CoA decarboxylase as a new transport enzyme for Na⁺ ions. The enzyme

Table 2 Effect of lonophores on ²²Na⁺ uptake into inverted membrane

	²² Na ⁺ uptake after incubation for		
	0.25 min	1 min	
Addition	(nmol per mg protein)		
None	1.14	0.63	
Valinomycin, 22 µM	2.22	1.24	
CCFP, 20 µM	1.56	0.65	
Nigericin, 22 µM	0.14	0.14	
None, methylmalonyl-			
CoA omitted	0.13	0.14	

See Fig. 1 legend for experimental conditions. CCFP, carbonylcyanide-p-trifluoromethoxy phenylhydrazone.

is closely related to the Na⁺ pump oxaloacetate decarboxylase⁶⁻⁸ and both enzymes constitute a new group of vectorial catalysts, converting the energy liberated by decarboxylation reactions into ion gradients. It is well known that the decarboxylation of certain 'energy-rich' carbonic acids is used as a driving force in biosynthesis, for example, in the biosynthesis of fatty acids and of phosphoenolpyruvate, where the decarboxylation of malonyl-CoA and oxaloacetate, respectively, shifts the equilibrium towards synthesis. The conversion of the energy of decarboxylation reactions into Na⁺ gradients by methylmalonyl-CoA decarboxylase and oxaloacetate decarboxylase, however, is a previously unknown biological use of decarboxylation energy, one closely related to other energy converting processes such as the formation of cation gradients by ATP hydrolysis.

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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Erratum

The article by F. W. Alt, N. Rosenberg, R. J. Casanova, E. Thomas and D. Baltimore, Nature 296, 325-331, was given the incorrect title. It should read 'Immunoglobulin heavy chain class switching and inducible expression in an Abelson murine leukaemia virus transformed cell line'.

Corrigenda

In the article 'Molecular cloning establishes proenkephalin as precursor of enkephalin-containing peptide' by U. Gubler et al., Nature 295, 206-208 (1982), in Fig. 2 the residue at position 20, shown as Asp, should be Asn, and the sequence around it Thr-Asp-Leu-Asn-Pro-Leu.

In the article 'The highly polymorphic region near the human insulin gene is composed of simple tandemly repeating sequences' by G. E. Bell et al., Nature 295, 31-35 (1982), the first three lines in Table 2 should read:

	Occurrences		
Sequence	Allele 1 (AHi1)	Allele 2 (λ H ₁ 2)	
a, ACAGGGGTGTGGGG	15 (44.1%)	24 (53.3%)	
b, ACAGGGGTCTGGGG	5 (14.7%)	6 (13.3%)	
c, ACAGGGGTCCTGGGG	7 (20 5%)	7 (15.6%)	

and the last two lines:

Arrangement of obsonucleotides

Allele 1 5' cdi aba aba dab aab aca aaa cea faa cgh ccc b 3' (34 repeats) Allele 2 5' cdi ifa faa aba baa aaf aba afa baa aac aaa cac baa afc ccb 3' (45

MATTERS ARISING

Thermomechanical inferences from deep seismic sounding sections

CHOWDHURY AND HARGRAVES1 recently interpreted a deep seismic sounding section, between Kavali and Udipi in India, which appears to show deep faults offsetting an essentially horizontal Mohorovičić discontinuity. Reflectors below the Moho seem to have lower and more uniform apparent dips than those above it, leading these authors to suggest that "at the time of its formation [before faulting] the Moho constituted a thermomechanical boundary between rigid [folding] crust and plastic [ductilely flowing] mantle". Although elements of this interpretation are questionable (notably that folding constitutes rigid behaviour), their subsequent conclusions ignore even more fundamental considerations regarding the rheology of solids: "Ductile flow requires that the then Moho level temperatures were close to the crustal solidus [of 1,000 °C for anhydrous granulite³]. Consequently, mean geothermal gradients must have been in the range 20-30 °C km⁻¹, two or three times those inferred for present day shields4"1.

The mere fact that a rock has been plastically deformed does not uniquely determine the temperature at which that deformation took place. Steady homogeneous flow in a solid is governed by a constitutive equation relating temperature, strain rate, and stress. For à rock subjected to uniaxial compression the equation relating the shortening rate $\dot{\epsilon}$ to the differential stress σ and absolute temperature T is typically of the form

$$\dot{\varepsilon} = A \exp(-Q/RT)f(\sigma)$$
 (1)

where O is an activation energy, R is the universal gas constant and A is a constant. The form of the function f depends on the dominant deformation mechanism(s), whereas the constants A and Q are determined by the minerals present, their grain size and geometry, and the activities of any volatile phases.

Solving equation (1) for the temperature gives

$$T = Q\{R \ln \left[Af(\sigma)/\dot{\epsilon}\right]\}^{-1}$$
 (2)

from which it is evident that the temperature required for flow can be reduced either by decreasing the strain rate or by increasing the stress. The validity of this conclusion is not limited to the case of uniaxial deformation; equations (1) and (2) can be extended to more general states of deformation, although the measures of strain rate and stress must be

Table 1 Temperature limits calculated using experimental flow laws

	Minimum temperature* (°C) [Range of mean geothermal gradients (°C km ⁻¹)]	Maximum temperature† (°C) [Range of mean geothermal gradients (°C km ⁻¹)]
Quartz	175 [3-6]	710 [10–24]
Diopside	440 [6-15]	975 [14–33]
Olivine	490 [7-16]	1,040 [15–35]

^{*} $\sigma = 2.5$ kbar, $\dot{\varepsilon} = 10^{-17}$ s⁻¹ † $\sigma = 20$ bar, $\dot{\varepsilon} = 10^{-12}$ s⁻¹.

appropriately generalized and the function $f(\sigma)$ may take a different form.

To determine unambiguously the temperature at which plastic deformation ceased (and at which the Moho formed in the Chowdhury-Hargraves model) we would need to know: (1) whether the deformation was homogeneous and steady; (2) what was the appropriate constitutive relation; and (3) what were the values of $\dot{\varepsilon}$ and σ just below the Moho when it formed. Condition (1) may be

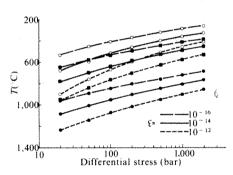


Fig. 1 Flow temperature as a function of differential stress for three strain rates. O, Quartz⁶; □, diabase⁷; ●, olivine⁵.

approximately satisfied for deep processes and geological time scales but requirements (2) and (3) cannot easily be met. If simplifying assumptions could be made about sub-Moho mineralogy and deformation mechanisms, an empirical flow law⁵⁻⁹ might be used. Similarly it could be assumed that currently estimated deep crustal or mantle flow stresses (several tens of bars to several kilobars) 10 and strain rates $(10^{-17}-10^{-12}\,\mathrm{s}^{-1})^{11,12}$ were applicable to the process of Moho formation. Using flow laws for quartz (ref. 6 and unpublished data), diopside⁸ and olivine⁹ aggregates along with the extreme values of stress and strain rate noted above, upper and lower bounds may be placed on flow temperature from equation (2). Table 1 shows these values together with their respective mean geothermal gradients for crustal thicknesses of 30-70 km.

Evidently Chowdhury and Hargraves' model places no real constraints on crustal thermal structure; in fact their near crustal solidus Moho formation temperature and corresponding geothermal gradients are nearly maximal values in a broad spectrum of possibilities. Unique determination of thermal structure by this method requires petrological and rheological constraints which are not generally available and cannot be assumed without prejudicing the results.

I thank W. M. Bruner and P. Koch for helpful suggestions.

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ROY CHOWDHURY AND HARGRAVES REPLY—Koch's argument pertains to the validity of an indirect consequence of our conclusion—the inferred geothermal gradient in Archaean times-without disputing the seismic evidence which prompted our overall speculation: that in Archaean times, the base of the crust was the base of the lithosphere 1. Nevertheless his point is well taken, because we had not considered rheology in our paper.

In essence, Koch is stating that if one takes the extreme estimates of the relevant rheological variables, one gets a wider range in the resulting geothermal gradient for our model than the 20-30 °C km⁻¹ we had inferred. These variables are (1) crustal composition and thickness, (2) stress and strain rate, (3) flow law. We do not dispute this, but would rather ask: what physical conditions are required to permit our interpretation of the seismic data in terms of the current understanding of the rheological properties of the crust and upper mantle?

It seems reasonable that the Archaean continental crust was differentiated much as it is today²—in which case anhydrous mafic granulites seem to be the likely material composing the lower crust. The "smearing out", we mentioned, would require this material to be ductile at its base. We used 38 km for the crustal thickness because it is the present average for the region under study. A 70-km thick Archaean crust seems unlikely as the Dharwar schist belts now exposed on the surface are only in the greenschist facies.

We do not believe that the possible range of values for the differential stress considered by Koch (tens to thousands of bars) is the probable range. Recent studies³ tend to suggest low stresses (10-100 bar) associated with contemporary asthenospheric flow. Similarly 10^{-14} s⁻¹ is considered to be a geologically representative strain rate4

Unfortunately rheological behaviour of geological materials in the above mentioned conditions is also not known uniquely. For example, the flow law of Goetze⁵ for olivine, reported to be valid below 2 kbar, yields a flow temperature of 1,235 °C for $\sigma = 20$ bar and $\varepsilon =$ 10⁻¹² s⁻¹ as compared with 1,040 °C from the law used by Koch. There is also the problem of extrapolating experimental values to low stresses and strain-rates.

Figure 1 shows the dependence of the flow temperature on differential stress for three compositions and three strain rates using flow laws for quartz⁶, diabase⁷ and olivine⁵. Clearly, the temperature is sensitive to chemical composition and the sensitivity increases as the differential stress decreases. Thus, if the smearing out of the base of the crust took place under relatively low stress fields (tens of bars), then the chemical composition becomes a particularly important factor in determining the actual temperature of the process. For a strain rate of $10^{-14} \, \mathrm{s}^{-1}$ we obtain a temperature range between 780 °C for diabase and 1,080 °C for olivine at 20 bar. If, as we had inferred, the structural discordance at 38 km is taken to indicate that the mantle (olivine) below was ductile and convecting, then, in the abovementioned stress and strain rate conditions, a temperature of ≥1,080 °C and a gradient ≥28 °C km⁻¹ are implied. As olivine appears stronger than other rock forming materials, this model requires that the lower crust must then have been ductile as well, and hence the base of the lithosphere must have been above the Moho-crust being defined in chemical terms without consideration of its rheological properties. If, on the other hand, the boundary marks that point in time when the descending brittle-ductile boundary in the crust reached its base. then in the same conditions, a temperature of 780 °C, and a gradient of

20 °C km⁻¹ is implied for a 'diabasic' type lower crust.

Note that the thermal gradients inferred above are entirely consistent with those claimed in our original paper, which were based on experimental evidence on the beginning of melting in silicate rocks⁸. With declining heat flow, model 1 would evolve to model 2, and in the interim, the lithosphere would include a ductile lower crust above a brittle upper mantle ("jelly sandwich") (J. Suppe, personal communication). Although model 1 may well have applied in the early Archaean, model 2 seems to be more reasonably associated with the onset of cratonic stability—which marks the end of the Archaean.

Clearly evolution of tectonic style in the Earth is associated with declining heat flow. Consideration of lithospheric evolution in rheological terms, with progressive descent of the brittle-ductile boundaries in both crust and mantle, as the Earth cooled, may help to explain the more abrupt changes.

We thank Brian Evans for discussions and computational help.

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Behaviour, paternity and testes size

IN a recent issue of Nature 1,2, Martin and May, and Harcourt et al. proposed a theory that behaviour, paternity and testes size are related. This hypothesis is interesting but suffers from the use of, as a model, species in which sperm production per unit of testes, sperm per ejaculate and other important considerations can only be surmised. Perhaps larger testes size is a result of more frequent copulations or larger testes stimulate males to copulate more frequently. Are sperm numbers per ejaculate well correlated to testis size in primates? The boar, ram and bull have roughly the same testis size but daily sperm production per gramme of testis varies greatly between them, and the number of sperm per ejaculate is 10-50 times greater in the boar than in the bull or ram.

The time of mating relative to ovulation seems to be more important than any other one factor in determining paternity when two males are used at an interval in the rabbit, pig and sheep^{3,4}. When matings or inseminations of two males are coincident, then the fertility of the male and numbers of sperm determine paternity. With all these complex interactions playing an important part, it seems overly simplistic to ascribe much to testes size.

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HARCOURT REPLIES-I agree, as stated in our paper¹, that not only are more data needed to prove fully the idea that relative testes size is related to breeding system via sperm competition but also that other factors influence the correlation, and also agree that the association between relative testes size and sperm output is largely an assumption. Nevertheless, I would argue that the correspondence between the data and predictions based on this assumption is so good that unless another hypothesis explains equally well the association between relative testes size and breeding system, ours (with its assumption) must stand as the best explanation of the correlation.

Four points can be usefully commented on in more detail. (1) Although data are lacking on, for example, sperm numbers per ejaculate, they are not totally absent, and what information there is supports the hypothesis 1,2

- (2) Dziuk writes about testes size per se; we consider relative testes size, that is, testes weight per unit body weight. The difference is extremely important, and the fact that the boar, with a greater relative testes size than the bull (and I believe the ram?), produces more sperm per ejaculate than does the bull or ram fits our theory and its assumption.
- (3) Timing of mating in relation to ovulation is of prime importance in determining paternity. However, primate males cannot judge precisely the time of ovulation. In this situation the male that inseminates the largest amounts of sperm over the longest periods will be at a competitive advantage when more than one male mates with the periovulatory female. Our argument is that to do this he requires a large volume of spermatogenic tissue and hence large testes. Dziuk implies just this

in his statement that "When matings...of two males are coincident. then...numbers of sperm determine paternity" (see, for example, ref. 3). Moreover, this statement made in relation to rabbit, pig and sheep supports our suggestion that the association between breeding system and relative testes size will be found in other mammalian orders.

(4) Finally, while the hypothesis relating breeding system to relative testes size (rather than to some more precise variable) is simple, the good agreement between the data and predictions based on the stated assumption implies that it is not simplistic.

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Hexose transport in hybrids between malignant and normal cells

IT HAS been reported by White et al.1 that a decreased K_m for hexose transport correlates with malignancy in matched pairs of hybrids between malignant and normal cells. Hexose transport was measured by the uptake of 0.1-5 mM 2deoxy[3H]glucose at 20 °C; in these conditions, uptake was found to be linear for at least 10 min. No doubt the authors are aware that sugars such as 2-deoxyglucose (or D-glucose) enter cells rapidly, soon (generally within <30 s at 25 °C) establish a steady state-at intracellular concentrations which can approach that in the medium-and are, in turn, phosphorylated at slower rates which persist linearly for several minutes^{2,3}. Within 5 min of exposing Novikoff² or Lettree⁴ cells (at 25 or 37 °C) to 2-deoxy[3H]glucose at concentrations of ≤0.2 mM, for example, >85% of the radioactivity associated with the cells is in the form of 2-deoxy-[3H]glucose-6-phosphate, whereas < 15% is present as an intracellular pool of free sugar.

In other words, it is likely that the ratelimiting step is phosphorylation, not transport, of the hexose and that what White et al. are measuring is the linear accumulation of 2-deoxy[3H]glucose-6phosphate in cells. While the use of a coupled reaction' to measure a preceding one is a commonly used biochemical technique, its validation does depend on confirmation that the second reaction is not rate-limiting. The article by White et

Table 1 Kinetic parameters of 3-O-methyl-Dglucose uptake in malignant and non-malignant cells

Cell type	Vmax	K _m
CBAT ₆ T ₆	325.1 ± 26.8	4.419 ± 0.520
fibroblasts	420.3 ± 90.8	4.868 ± 1.214
PG19	106.6 ± 5.1	2.114 ± 0.172
	116.6 ± 18.2	2.211 ± 0.460
1Acn2	331.2 ± 40.2	8.495 ± 1.282
1Acn1TG	107.6 ± 7.3	2.771 ± 0.241

The uptake of 3-O-methyl-D-glucose measured by the technique previously described1 for 2-deoxyglucose with the following modifications: (1) 3-O-methyl-D[1-3H]glucose (0.1-5.0 mM; 10-500 mCi mmol-1) was added to the cells in a volume of 250 µl and its uptake terminated by washing the cells twice with 1 ml of ice-cold phosphate-buffered saline.
(2) At 20 °C, uptake of 3-O-methylglucose is linear for only 1-2 min, so that correspondingly shorter incubation times were necessary. Kinetic constants were calculated as before from the linear portions of the uptake plots.

al,1, however, contains no mention of the phosphorylation reaction, let alone measurement of intracellular 2-deoxy[3H]glucose and 2-deoxy[3H]glucose-6-phosphate. Moreover, some authors have suggested that it is sugar kinases (not sugar transport per se) that are different in transformed and normal cells⁵

Although the results of White et al.1 clearly show that malignant cells take up hexoses at low concentrations better than their non-malignant homologues, the claim of these authors that their "findings leave little doubt that malignancy is closely linked to a decrease in the $K_{\rm m}$ of the membrane hexose transport system" seems as yet unjustified.

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WHITE ETAL. REPLY—Since our paper¹ was submitted, we have succeeded in establishing conditions in which the

uptake of 3-O-methyl-D-glucose in our cells is sufficiently linear to permit formal kinetic analysis. This has enabled us to repeat our experiments using a glucose analogue that is not phosphorylated. The $V_{\rm max}$ values for 3-O-methyl-D-glucose uptake are comparable with those for 2deoxyglucose uptake, but the K_m values are two to four times higher, indicating that 3-O-methyl-D-glucose is transported less efficiently than 2-deoxyglucose or glucose. Nonetheless, the reduction in K_m that we previously described for 2-deoxyglucose uptake also occurs with 3-Omethyl-D-glucose.

Table 1 gives representative values for CBAT₆T₆ mouse fibroblasts, PG19 malignant mouse melanoma cells, 1Acn2, a human hybrid cell line in which malignancy is suppressed and 1Acn1TG, a malignant segregant derived from this hybrid. The reduction in K_m that we described previously is thus not primarily determined by the phosphorylation reaction, but by the flux of hexose across the cell membrane. Measurements we have made of the hexokinase activity in cell homogenates indicate that there is no correlation between hexokinase activity and malignancy.

Our conclusion that malignancy is closely linked to a decrease in the K_m of the membrane hexose transport system stands.

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nature

15 April 1982

The Falklands from below

Argentina's seizure of the Falkland Islands, a test of honour for Britain that may cost it the Thatcher government, is also a test of President Reagan's peacekeeping abilities.

Falklands; one was dry, one had gas and one produced 5,000 barrels a day in test drilling. Nearer the Argentine coast, a shell well showed 3,000 barrels a day. So the theory goes, Argentina, beset by economic woes, seized the Falklands to assure supply of a new oil well.

For the most part the oil theory is rubbish. Argentina is very

What is to happen to the great white continent to the south, Antarctica, which has no voters to cast down governments or crowds to wave approving placards in the streets. It may seem trivial to talk about obscure Antarctica, currently a haven for scientists of many nations, its antique sovereignty disputes, in this solemn moment. But the Falklands fiasco has shown how rapidly the obscure far south can be suddenly important. Whether it brings war between Argentina and Britain or a stalemate and negotiations, the dispute has three serious implications for the Antarctic, a region that starts at 60 degrees south latitude, beginning south of the Falklands and South Georgia, the southernmost island taken by Argentina on its weekend spree.

First there is the extreme vulnerability of human settlements in the far south to military takeover. Most of the Antarctic stations south of South America consist of a gaggle of huts nestled against steep black mountains and glacier snouts, the men in bright coloured parkas providing the only colour in a nearly lifeless landscape — except for the thousands of screeching birds — the huts bristle with antennas, but except for a passing ship, the only communication with the outside comes by often poor radio.

The settlements in the Falklands and at Grytviken in South Georgia, different only in that they had real buildings and greener hills, were easily overwhelmed by Argentine forces coming from the mainland a few hundred miles away. It would be harder but not much for a Latin-based military force (or one based in the Falklands) to make the 800 mile crossing of the Drake passage and seize the remaining four British stations there — although such a seizure would be a willful violation of the 1957 Antarctic Treaty that demilitarizes the region, and to which both Britain and Argentina belong. It is unlikely that Argentina would risk the enormous international opprobrium that would follow such a move, but actions in the Falklands remind us how fragile are these sparse southern stations, and how valuable is the Antarctic Treaty.

Indeed, there is a real question as to whether the British Antarctic Survey, which runs the UK's science programme in the Antarctic and until now staged its far-south operations from Stanley and Grytviken, can continue to operate, although it has contingency plans (see page 593). At present, perhaps two dozen British scientists and technical personnel are either being held or are at large on South Georgia — a place whose mountainous hinterland is so impassable that one needs an axe to cut steps in the ice to cross its inland glaciers.

The second implication of the Argentine seizure is oil. According to a report presented in January by Washington energy consultant Lawrence Goldmuntz, the offshore region between Argentina and the Falkand Islands is one of four major "horizons" in the world outside the Middle East capable of producing oil on the scale of the North Sea. Recently, Exxon drilled three wells about midway between the coast and the

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nearly self-sufficient in oil, and by inflaming a dispute to ownership of the Falklands, it would certainly not encourage major oil companies to take out leases. Despite having one of the widest, potentially richest continental shelves in the world, big oil companies have not exactly flocked to do business with Argentina. While its own near-shore shelf and Tierra Del Fuego remain undeveloped, it is hard to justify a push in the unexplored far-off Falklands. Yet the dream of oil wealth may have played a subliminal role in the seizure of the Falklands, and will play a key role in the disposition of Antarctica. Just to the south in the Weddell Sea (claimed by Argentina, Britain and Chile) sedimentary rocks are many kilometres deep while the shelf itself is larger than all of Venezuela. Argentina, West Germany, Norway and even the Soviets have been surveying the geology of the region, to get basic geological data as well as some idea of its oil potential.

The Falklands experience shows that arcane notions of national sovereignty, when combined with the dream of great oil potential, can be an explosive combination. It is easy to laugh at the obscure theories under which seven nations, and potentially several others, claim national sovereignty in Antarctica. It is also easy to dismiss the Antarctic's oil potential as purely hypothetical, as no serious exploration has been done. But the Falklands experience also shows that, nonetheless, the potential for international mischief is great.

The third and most important implication of the Argentine seizure of the Falklands is that it strains international relations at the far south at a delicate moment in Antarctic diplomacy. Argentina itself convened last year the first major international discussion by the 14 Antarctic Treaty powers of how they should dispose of Antarctic minerals — a subject not covered by the original treaty. A second meeting will be held in New Zealand in June, and the list of full parties to the treaty includes many of the key players in the Falkland Islands dispute. After the Buenos Aires meeting, the group showed some promise of being able peacefully to resolve this contentious issue — going as it does, to the heart of the sovereignty dispute. But if Britain and Argentina must use the New Zealand meeting to pound shoes on the table over the Falklands, the result could be disaster for Antarctica.

Clearly, the boundary of the Antarctic Treaty area at 60 degrees south latitude, which in reality is only a windy, stormy sea, should be treated as an iron wall diplomatically by Argentina, Britain and the other Antarctic Treaty nations. Every effort must be made to keep the dispute — no matter where it stands then — from spilling over into the Antarctic question. This will be difficult indeed, but the parties would do well to remember that the Falkland Islands are only 6,200 square miles, about the size of Connecticut or Wales. The Antarctic area, besides comprising an entire continent, one fifth of the world's ocean water by volume, and its largest potential fishery, contains one fifteenth of the Earth's surface. It would be a shame if a dispute over these tiny islands, however serious, were to spoil it.

Graduate students galore

The government document on British graduate education fails to grasp conspicuous nettles.

The poor, it is said, are always with us. So too are graduate students, called postgraduate students in the United Kingdom. And the analogy with the poor is not misplaced. One of the strongest threads running through the report of the working party on postgraduate education in Britain set up by the Advisory Board for the Research Councils in 1979 and published last week is the evidence it provides for the impoverishment of graduate students while following their courses and the poor financial prospects which await them afterwards. This is one reason why any thorough study of the arrangements for organizing and supporting postgraduate students must also ask whether the whole enterprise is worthwhile. This study*, which bears the unmistakable stamp of its chairman Sir Peter Swinnerton-Dyer, has done this and more. While concluding that graduate students (a comparatively recent innovation in British universities) have come to stay, the working party has also provided a view of the working of the British system of higher education that could not otherwise have been obtained.

Part of the trouble with graduate education is that it does not fit in easily with the rules that the administrators of higher education have developed for deciding how much money should be spent on supporting universities. So much is evident from the length of time taken after first graduation for different students to qualify for a PhD or some equivalent research degree. Why should some students take only two years, and others five or more? What, in any case, is signified by a PhD degree? Does it denote competence in research, or extra knowledge in some special field or is it merely a sign that its usually proud possessor has qualified by earlier performance for financial support or has been sought after as a research assistant by some supervisor looking for help with his own research? Because circumstances may make each of these readings valid, it is natural that administrators should be at a loss to know how much public support should be devoted to graduate education. These are some of the perplexing questions that seem to have prompted this working-party's study.

So far as they go, the recommendations of the working party are sensible enough. Yes indeed, it says, the time has come for the British government to give up the attempt that its predecessors have made ever since the Second World War to justify spending on university research by means of some calculation that there is a direct link between research expenditure and the improvement of national prosperity. Moreover, the argument goes, it is also impossible, or at least impracticable, to relate public expenditure on the support of graduate students to the needs of employers in different fields. For what employers consider to be their needs are moving targets. The only substantial exception to this chastening truth, the report concludes, is that there may be a continuing need for short-term graduate courses in fields such as computer science or toxicology, where shortages of skilled manpower have recently become apparent — and where they may nevertheless disappear without reason any time. So, the argument goes, the research councils should be quicker off the mark both in their willingness to support courses such as these, if necessary at universities chosen centrally — and then more ready than they are at present to close them down. Nobody will dissent from these conclusions. How readily will the research councils adopt them?

In at least one important respect, however, it is unfortunate that the working party has not gone further. As things are, the chief source of support for graduate students at British universities are the research studentships handed out each year by the research councils. The chief recipients of these grants are not, however, students but the departments which eventually house them and which are, within the rules, then empowered to select the students they consider to be best qualified for a career in research. The working party recommends that the research councils should be more vigilant than in the past in matching the

quotas to the reputations of their dependent departments research — and that departments whose students seem to take to long to qualify for their degrees should be penalized. The snag that in present circumstances, the principle of the quota syste needs radical re-examination. When the whole pattern of Briti higher education is being changed in such a way that sor university departments will become centres of excellence at others in the same field will have virtually to abandon hope of evbeing strong in research, is it equitable or even wise that the appointment of state-subsidized graduate students should be be entirely to the lucky (and no doubt deserving) university departments? Should there not at least be some device for making sure that promising people from less favoured universities have chance?

The working party might also have profitably gone further in i examination of the character and quality of graduate education British universities. It seems to have been mesmerized by the publicity given in recent years to the sharp difference between th tenor of PhD courses in the natural and the social sciences. In th natural sciences, it seems to be accepted that in three years c thereabouts, a person can pick up a sense of how to conduct research and also to carry out a substantial piece of work, but the in the social sciences it takes three years to acquire "researc training". So, the working party argues, the social science departments of the United Kingdom should tailor their graduat courses to the acquisition of research skills; whether or no successful students are then given a PhD degree become irrelevant. But how does it arise that in the United States an elsewhere, the normal pattern of graduate courses in the natura sciences includes a more substantial element of research trainin ("taught courses" and all that) and often a longer time i harness? Has the committee plumped for three years as th normal period of graduate education leading to a PhD out of deference to the British Government's wish to economize where i can, and without sufficient regard for the quality of graduat education?

The economic value to students of graduate education in British circumstances is exceedingly problematical. Starting salaries for PhD graduates tend to be lower than those of people who find jobs immediately after their first degree, no doub because substantial proportions of PhD graduates choose to take jobs in academic life. The working party's survey of employmen among British graduates of various kinds does not pretend to throw light on economic prospects in the longer term, which is entirely forgivable. But the data that it has uncovered are sufficient to give the lie to the now common supposition in Britair that graduate education is an extravagant luxury, a way in which perpetual students may forsend the harsh realities of the rea world for a few years — and often at the public expense. So the working party is right to flirt with the notion that the public stipends of chosen graduate students should be linked not with the grants paid to publicly-supported undergraduates but with those of research assistants doing real jobs in a laboratory environment.

Fine tuning such as this will help to make the present system function more efficiently, but will not by itself revivify graduate education in British universities. Traditionally, PhD courses have been devices for selecting future academics but, when universities are shedding staff, there are many fewer opportunities than aspirants. Is it then surprising that many graduate students become dispirited, and give up along the way? So the most urgent need is that universities should acknowledge that even the most talented graduate students will need to find other than academic jobs. Industrially related graduate courses, such as those sponsored by the Science and Engineering Research Council are valuable but will not suit every need. So should not universities pay more attention to the fate (as well as the selection) of their graduate students? And should not more of them acknowledge that there is a crying need for more than one-year graduate courses designed deliberately to round off the standard three-year first degree course by the acquisition of some employable skill?

*Report of the Working Party on Postgraduate Education, Cmnd. 8537, HMSO, £7.00.

Antarctic research hit by crisis

BAS counts the cost of island invasion

The present dispute between the United Kingdom and Argentina over the Falkland Islands has raised fears about the future of the British Antarctic Survey. But according to the survey's director, Dr Dick Laws, the invasion of the islands by Argentina came too late to have much effect on this season's work, with bases on the Antarctic continent already preparing for the long isolation imposed by the austral winter.

The British Antarctic Survey depends on the Falkland Islands to maintain contact with its research stations. The US National Science Foundation has agreed to provide a temporary commercial relay service via its base on the Antarctic peninsula, Palmer, stressing that it would only pass on messages of a scientific nature and would not handle those dealing with "political matters". In the long term, though, the communications problem could be resolved by setting up a new relay station, probably at the existing British base on Signy Island, in the South Orkneys. The Falklands are also used by the survey as a supply base, and although not indispensable, loss of those facilities would undoubtedly add to the difficulty and cost of operations. Alternative refuelling facilities within easy reach of the British bases are in the Magellan Straits, but so far Chile has not offered use of these.

Direct confrontation between the survey and the Argentinians has so far only occurred on South Georgia, where the commander of Grytviken base is also the senior representative of the Falkland Islands government. Grytviken, base for 26 of the survey's scientists at the time, had earlier been the scene of fighting between Royal Marines and Argentinian forces, although it is not yet known whether the base suffered any damage. Operations at Grytviken were in any case due to be reduced as from this month following budget cuts, despite pressure to maintain a British presence there. South Georgia has recently become a centre for work on krill, whose study as the dominant planktonic organism in the Southern Ocean is important for the exploitation of the Antarctic seas.

These problems come at the end of a troubled season for the British Antarctic Survey. In November the survey lost its two aircraft in hurricane-force winds at Rothera Station, resulting in the cancellation of virtually all Earth science projects, which rely heavily on air support of field parties. Damage to a De Havilland Twin Otter aircraft and the research vessel



South Georgia, showing the British Antarctic Survey's base at Grytviken (left) and the former whaling station (foreground).

John Biscoe the previous year had already meant abandoning that season's marine biological work, and cutting short other research programmes.

However, the future for what is widely acknowledged to be one of the most costeffective research organizations operating in the Antarctic, is not entirely bleak. The Natural Environment Research Council, who provide the survey's annual funds of £5.6 million, have provided an additional £1.8 million for the purchase of two new aircraft, and £1.3 million for the rebuilding of an important station at Halley Bay, now nearly ten years old. Collaborative research with West Germany is another route that the survey has been exploring to keep within its budget.

The Argentinian action will probably not immediately affect the activities of other nations in the area, which include the United States, the Soviet Union, Poland, Chile and Argentina itself, as none of these rely upon the Falklands for their communications or supplies. Although Argentina's own research efforts have been extremely limited, it is at present collaborating with the French in glaciological work, and plans exist to site a ground station for the first European Space Agency remote sensing satellite to be put into polar orbit, at the Argentinian base of Marambio.

Clearly the Falklands invasion is going to affect Argentina's standing amongst the Antarctic Treaty nations, due to meet in Hobart in two months' time to discuss the future exploitation of a region with a hitherto exemplary history of peace and international cooperation. David Millar

Graduate students on shorter lease

British postgraduates take too long to complete their PhD theses, according to the report of a working party on postgraduate education published last week. Blame is laid primarily on the universities, although the research councils are also criticized for not taking matters more firmly in hand.

The working party, set up in 1979 by the Advisory Board for the Research Councils, found wide discrepancies in PhD submission rates between universities and between the natural and social sciences. Its recommendation that the research councils award postgraduate quotas on the basis of a department's past completion record is being taken up by the Science and Engin-

eering Research Council (SERC) in deciding quotas for 1982-83. SERC has also published a pecking order of universities based on PhD completion success which is intended to shame universities into remedial action.

The working party was set up two years ago under the chairmanship of Sir Peter Swinnerton-Dyer, then vice-chancellor of the University of Cambridge, to advise on postgraduate education policy. It has managed to comply with its remit to assess the ways in which research councils make awards to universities and particular disciplines and whether sufficient postgraduates of the right quality are produced. But the task of determining

whether postgraduate education is meeting manpower needs proved too complicated, chiefly because postgraduates often find employment in areas outside their disciplines of study and employers are usually unable to indicate precisely their future needs at postgraduate level.

The working party report concludes that postgraduate education should continue much at the present level, but suggests that the Natural Environment and Social Science Research Councils should take a leaf out of SERC's book by encouraging specific postgraduate advanced courses in subjects for which there is a growing need.

PhD success rates of SERC-supported students by subject

	100
Subject	Success rate (%)
Biology	68
Chemistry	75
Physics	61
Maths	56
Astronomy	58
Engineering	52
Social science	39
Average	58.4

The data are for 1974 and 1975 starts, and were collected in July 1980-February 1981.

Strangely enough, however, unemployment rates are greater for those who have followed advanced courses than for those who have completed research studentships and, in the same breath, the working party also recommends that SERC should move towards research rather than advanced course studentships. It points to the reluctance to wind down courses once a specific manpower need has been met and SERC's difficulty in finding students of sufficiently high calibre to fill the places reserved for advanced engineering courses.

The universities come in for the worst drubbing. Poor PhD completion rates are attributed mainly to the morale of particular departments and the lack of adequate supervision. Poor supervision, the report says, can lead students to an unwise choice of research topics and can result in inadequate training in the techniques of research.

On the substantial differences in PhD completion rate between the natural and social sciences, the report says that there are inherent differences between the subjects. Thus social science students concerned that their first work should be a magnum opus, have a greater tendency to take on research topics which are far too ambitious to be completed within the normal three years. Perhaps, therefore, it is no surprise that 54 per cent of those embarking on PhDs in the natural sciences in 1975 had completed within five years compared with only 31 per cent in the social sciences.

Nevertheless, the working party finds the PhD completion rates in both disciplines "wholly unsatisfactory". The criteria for a PhD may have become more stringent in recent years, says the report, leading some students to take up topics unsuitable for a three-year study. Another

Average salary of graduates by occupation and highest postgraduate qualification achieved (1979-80)

Engineering and technolog. None	
	£5,520
MA	£5,700
PhD	£5,085
Science	
None	£5,350
MA	£5,970
PhD	£4,855
Social studies	
None	£5,810
MA	£5,970
PhD *	£5,000

problem is that students from small departments can lose motivation, especially if inadequately supervised. It therefore recommends that the research councils concentrate their postgraduate awards in larger departments where students may be attached to groups of experienced researchers working on a particular problem, and that supervisors should take more care to fulfil their responsibilities. The aim should be to complete research work within three years and to submit a final thesis before the end of the fourth.

The working party's report recommends several remedial actions. Universities should monitor research students at the end of their first year of postgraduate study and relegate those unsuited for research to MSc courses. Both outside academic institutions and industry should have a greater role in the choice of an individual's research topic. Finally, the research councils should review their postgraduate policies every four years, taking into account representations from potential postgraduate employers. Judy Redfearn

University research staff

Postdocs' plight

More than a quarter of the academic staff at the University of Bristol are contract researchers — postdoctoral students who in years gone by would now be on the academic staff. This is one of the more startling conclusions of a detailed survey of Bristol academics just completed by the lecturers' trade union the Association of University Teachers (AUT).

AUT claims that this is the most comprehensive study of university employment yet undertaken in Britain, and it reveals the frightening extent to which universities appear to be relying on labour which, if the research councils firmly apply their rules of cutting off postdoctoral support after, say, six years, could rapidly be lost. Nearly two-thirds of the contract researchers have less than a year of their present contract to run, the survey showed. Thus the university system could shrink without the government lifting a finger.

Naturally, AUT is most concerned about the individuals who may lose their jobs. A third are over 30 years old, one in five has dependent children, and nearly 40 per cent of researchers who have either masters or PhD degrees have no "academic status" (granting them voting and library rights, for example) in the university.

The survey, says AUT, "shatters the myth" that contract research can be regarded as a brief step on the way to an academic post. Nine per cent of the researchers surveyed had had four or more contracts, and 34 per cent, two or three. Twenty-eight per cent of the contract researchers had already been in research

Med comes clean

Political bickering between the 18 countries surrounding Mediterranean has not stopped the initialling of the fourth protocol and arm of the United Nations Environment Programme's (UNEP) plan to save the Mediterranean from further environmental damage. Mediterranean action plan was launched at the Barcelona convention in 1976, and the latest agreement obliges the signatory countries to set up coastal protected zones. The aim is not just to preserve a representative cross-section of Mediterranean ecosystems but also to foster commercially viable farms for oysters, lobsters and other shell fish.

The 1982-84 budget of \$7 million will mostly be shouldered by Italy, France and Spain, with \$400,000 from the EEC itself. A major spur for this spending is the concern that environmental dangers will affect the tourist trade. From a scientific point of view the protected areas will be useful for baseline studies of ecosystems to see whether coastal waters are getting worse or better.

Among the Mediterranean species in danger of extinction are the monk seal, marine turtle, European otter, Dalmatian pelican, peregrine falcon, oysters, mussels, the Iberian midwife



toad, the pied kingfisher and the spectacled salamander. UNEP hopes that the present number of 15 marine parks and reserves will eventually grow to at least 100.

Jasper Becker

In a report on the survey addressed to the university administration, AUT makes thirteen recommendations, most of which entail effectively putting contract workers on the staff.

According to AUT, the contract researchers should be given academic status, salary scales and a career structure. The university should have a contingency fund to support contract researchers between contracts, and it should "exert pressure" on the research councils to develop "a more positive approach" to the employment conditions associated with their contracts (which support 60 per cent of the researchers concerned). AUT also recommended that the universities should abolish waivers in contracts, which absolve them from paying redundancy pay and remove the right of appeal against unfair dismissal.

• In a survey of the employment prospects for postgraduate astronomers in Britain, the Royal Astronomical Society (RAS) has come to conclusions which broadly confirm the AUT study at Bristol (see above). Some 42 per cent of respondents were in the fourth or later year of a postdoctoral appointment. And 40 per cent of non-tenured astronomers are over the age of 30. RAS respondents were also asked if their degrees and research enhanced their employment prospects. The proportion saying yes fell with increasing qualification, from 79 per cent for the first degree to only 56 per cent for postdoctoral Robert Walgate research.

British Aerospace

Looking good

A new mood of optimism prevails at the space and communications division of British Aerospace. Not surprisingly, the company is delighted at last month's news that the Hughes Aircraft company is to build up to 16 Intelsat-VI telecommunications satellites: as principal subcontractor British Aerospace stands to win orders worth up to £50 million over the next seven years. And having also won the contract to build the European Space Agency's next generation telecommunications satellite, L-sat, the company is now said to be working to capacity.

The arrangement with Hughes over Intelsat VI, however, is just one step towards what is hoped will develop into wider cooperation. British Aerospace, through its participation in the programmes of the European Space Agency, believes that its credibility is now well established in Europe but that it will never enter the US market alone. Thus the link with Hughes is cherished.

The Intelsat VI contract could be worth \$1,600 million to Hughes if options on all 16 spacecraft are taken. The plan is to build five spacecraft initially for launch aboard Ariane or the space shuttle from 1986 onwards. Hughes has taken many of the prizes in the Intelsat developments — only

contracts for Intelsat III and V were awarded to other companies, TRW and Ford Aerospace.

The capital for the Intelsat VI development will be raised by the 106 member organizations which consist mainly of national telecommunications services. The largest shareholders are the United States, with 20 per cent of the shares, and British Telecom which holds a 13 per cent share. Intelsat VI will carry 33,000 voice and four television channels, more than twice the capacity of Intelsat V. The design, which incorporates a satellite-switched time division multiple access capability for digital transmission, may be up-graded to 100,000 voice channels later.

Judy Redfearn

UK cancer research

Marking time

Britain's leading cancer research laboratory, the charitably supported Imperial Cancer Research Fund (ICRF), claims it is running short of cash — despite last year's income being the largest ever. New fund-raising mechanisms are being sought, including high-street shops and — in the long term — biotechnology.

Where has all the money gone? ICRF was criticized a few years ago for being overcautious, salting away charitable pennies in an endowment fund while spending too little on current research (only 32 per cent of its income in 1971). Now ICRF claims the balance has gone too far the other way: 58 per cent (£10 million out of £17 million raised) was spent on research in 1981, and savings have fallen correspondingly from 44 per cent in 1981 to 20 per cent last year. This year there is talk at ICRF of cuts: perhaps a loss of six staff, whereas over the past three years staffing levels have risen by 50 per cent.

The recent expansion has been the work of Dr Walter Bodmer, present research director, and his deputy Dr Michael Crumpton, but the spending seems to have overshot the mark. Government spending cuts are blamed. Not that the government supports ICRF, but cuts in the Health Service and in universities have meant that research councils, and bodies such as ICRF, have found themselves spending more than expected to provide basic services when they collaborate with hospital clinics or university laboratories.

ICRF commitments to support a new cyclotron for neutron therapy at Clatterbridge Hospital near Liverpool (the ICRF contribution is £3 million), and the building of a new £12-million laboratory site at South Mimms in north London, to replace the Mill Hill site (whose lease runs out in a few years' time), have also eaten into the budget.

The remedy will be to halt the expansion of the research budget and to attempt to raise more money by appeal — difficult in the present economic climate — and by

other means. One plan is to open shops selling second-hand clothes and bric-a-brac along the lines of the operation run by Oxfam, the famine relief organization.

ICRF is also making its entry into biotechnology. The prime objective is not to make money, says Dr Bodmer, although money would clearly be welcome if it came along. Rather, the principle will be to make commercial agreements which will speed the distribution of clinically or experimentally useful products (such as monoclonal antibodies) but to avoid "inappropriate" exploitation of the products outside ICRF control. The fund has engaged patent and commercial agents to help in this activity.

To speed this work, the South Mimms site (which should have opened in 1986) will have what is described as a "pilot plant" for large-scale cultures, which will have the double function of supplying the ICRF laboratories with materials and of testing cultures for potential industrial scale-up.

In the rest of its space, South Mimms will house the research services presently at Mill Hill and at the main ICRF laboratory in Lincoln's Inn Fields. After all the shuffling, space would be left at Lincoln's Inn Fields in 1985–86 for perhaps two new groups. Oncogenes and growth factors are among the research areas that are under consideration.

Robert Walgate

Biotechnology

Endorphin now

University College London last week announced a new joint venture in biotechnology that is more significant as a sign of the times than for the sum of money involved. Nevertheless, for Professor Brian Rabin and Dr Peter Butterworth of the college's biochemistry department, the \$240,000 contract to clone pancreatic endorphin is a godsend when the British government's economies in higher education are beginning to bite.

The contract with University College comes from Endorphin Inc., a company founded in Seattle in February. The company is equipped with venture capital, a patent and a president, Professor John Houck, who uses the money he has raised to support his own laboratories at the Virginia Mason Research Center in Seattle and now to support the University College project.

Professor Houck founded the company on the basis of his discovery that pancreatic tissue contains a hormone of potential therapeutic value. The hormone, not yet completely characterized, is related to the endorphins which also have therapeutic potential, particularly as analgesics. According to Professor Houck, however, whereas intravenously administered endorphins cannot readily reach the brain in an active form, the pancreatic endorphin can do so.

To prove the point and to prepare the way for clinical trials, Endorphin Inc. now needs a quantity of pancreatic endorphin that would be difficult to purify from pig pancreas but should be well within the production capacity of bacteria into which the relevant gene has been cloned.

The choice of University College for this task seems in large part to have been based on the fact that Professor Houck has been a visiting fellow there for several years. It may also have been cheaper to contract for the work in the United Kingdom than in the United States. But for the department of biochemistry, whose annual support from the university for overheads has been cut from £80,000 to £60,000 (an even more drastic cut in real terms), the contract allows at least one project to be carried out

in style.

Dr Butterworth claims that the normal funds available to university departments are now simply insufficient to buy the radioisotopes and restriction enzymes necessary for most gene cloning projects. Nor is there a wealth of British venture capital waiting to help out. Since matters are likely to get worse rather than better he and Professor Rabin hope that the contract from Endorphin Inc. will be followed by others

They hope also that the gene cloning for Endorphin Inc. is successful enough for the contract to be renewed in two years' time and that pancreatic endorphin eventually reaches the market. If it does University College stands to gain a one per cent royalty on all sales.

Peter Newmark

Europe leads on sequences

Europe appears, for a change, to have beaten the United States to the mark. The European Molecular Biology Laboratory (EMBL) at Heidelberg has announced the formation of a nucleotide sequence library, while the National Institutes of Health (NIH) in Washington are still deliberating the question.

Not that there is any sense of competition. Greg Hamm, manager of the Heidelberg library, is "still talking" to NIH, and wishes to cooperate with any system that NIH may set up. But EMBL was under pressure from European scientists to start now, before sequence data were irretrievably lost. EMBL is not attempting to become the sole manager of world sequence data, Hamm insists.

The question now is how to collect the data efficiently. The 600,000 nucleotides already logged at Heidelberg (they are freely available on magnetic tape) have taken an immense effort to collect. This is largely because journals do not use the clearest of systems for displaying the sequences, say Hamm and Professor Ken Murray in a letter recently sent to journal editors.

Hamm and Murray are recommending that journals should insist on the separate

submission of sequence data to the EMBL library, preferably in a format specified by EMBL, and if possible in computer-readable form. Some of these requirements, however, conflict with common format in journals. For example, codons should be presented in tens or fifteens of nucleotides rather than the triplets which correspond to the translation of the code into amino acids, says EMBL, to reduce counting errors; and the marking of reading frames and alignment of comparable sequences should be avoided.

According to Hamm, however, such requirements are only important for the easy transferral of the data into the EMBL computer: the printed format in a journal, which is intended to present the data heuristically, emphasizing the significance of regions of a sequence, could be quite different — provided EMBL were sent the uncluttered sequence according to their own guidelines.

So far, only the Journal of Molecular Biology, edited by Sir John Kendrew, has said it will implement the EMBL proposals in full. Nucleic Acids Research is said to be "basically positive", but discussing details. Nature is considering the matter.

Robert Walgate

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MMIG20 MUS.MUSCUL.IG.MOPC41; DNA; 350BP.
 82.01.01 (first entry)
 First two exons in immunoglobulin light chain genes from cell line \ensuremath{\mathsf{MOPC41}},
 differentiated gene; immunoglobulin.
 Mus musculus (house mouse, souris domestique, Hausmaus)
Eukaryota; Metazoa; Chordata; Vertebrata; Tetrapoda;
Mammalia; Eutheria; Rodentia.
               (bases 1-350)
 Altenburger W., Steinmetz M., Zachau H.G.;
"Functional and non-functional joining in immunoglobulin light
chain genes of a mouse myeloma";
Nature 287:603-607(1980).
 Kev
                         From
                                              Ťo
                                                                 Description
                           126
                                            176
                                                                 first exon (leader peptide
second exon (variable part
                           303
                                      >350
Sequence 350bp: 80 A; 82 C; 122 T; 66 G. CGTGGGGG CGTGGGCAT CGTAATGAT TOGATACCC TAACTGCT CGTTAATGAT TOGATACCC TAACTGCT GGCTAGCGC CTTCTTTATA TAACAGTCAA ACATACCCT TGCCATTGCT ATTCGAGTCA GGACTCAGCA TGGACATGAG GGCTCCTGCA CAGATTTTTO CCTTCTTTT CATCGTTAAACTTA AAATTGGGAA TTTCCAACTG TTTCCAACTG TOGTTAGTGT TCACTGCGCAT TTGGAGGGTA TTCATTATTG TCACTGCCAC TAGGTACCAG ATTGGACAC CAGATGACCA AGCCTCCACT CTCCTTATCT
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A sample entry from the EMBL sequence data library, specifying the source of the DNA as closely as possible, giving a reference and listing special features of the sequence in a table (in this case showing the exons). The original is approximately 6 inches wide.

Polish universities

Trial by proxy

New York

The International Council for the Future of the University (ICFU), a New York based organization of some 300 academics from 21 countries, committed to the defence of Western university traditions, has launched a study of the current situation in Polish universities. This marks a major change in ICFU policy. Previous studies have dealt with Western European countries (Sweden, Italy, West Germany) to which it was possible to send working parties and in which the ICFU already possessed members who could contribute first-hand knowledge of recent events. But with no Polish members of ICFU, and with the continuation of martial law the possibility of an ICFU commission visiting Poland in the near future seems remote.

The inaugural meeting for the new study, which was held in New York earlier this month, had to do the best it could with the testimonies of visitors, including Andrzej Kaminski, a mediaeval historian who left Poland before the foundation of Solidarity, two representatives of the banned Independent Students' Union (NZS) and Wojciech Karpinski, a political scientist and former activist now at Yale. Inevitably, their accounts of Polish university history did not cover the period of martial law. The most up-to-date material, including the martial law regulations governing the universities (see Nature 31 January, p.181), came from the floor. A detailed discussion of the events which followed the military take-over was deferred to a meeting to be held in Paris later this year.

More incongruously, the reforms urged and in part initiated in Polish universities during the 16 months of "renewal" (between 30 August 1980 and 12 December 1981), had been largely directed towards greater democracy ranging from student representation on the academic councils of universities to the possible election of a Minister of Higher Education by the university rectors - a proposal put forward only a few days before the imposition of martial law, and one which was later described by Deputy Prime Minister Mieczyslaw Rakowski as "the academics running amok". This trend contrasts sharply with the attitude of ICFU, which has tended to see too much democracy as a potential threat. But this divergence of views may make it easier for the Polish authorities to accept the impartiality of the proposed ICFU study. If the Polish propagandists look at the track record of ICFU it might find it difficult to make out a plausible case that ICFU is manipulated by Solidarity extremists abroad, when for the past ten years it has systematically deplored many of the reforms since advocated by Solidarity. Vera Rich

Data banks

Right to privacy

Brussels

Following the adoption of the European Parliament's resolution on the rights of the individual with respect to data processing, it now seems that the European Commission may well start work on drafting legislation to protect the confidentiality of information held in data banks. The European Parliament's resolution at the plenary session in Strasbourg last month has particular relevance for the United Kingdom which has so far shied away from tackling the controversial issues of the freedom or protection of information.

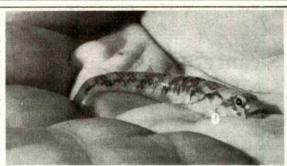
Respect for the individual's privacy has become a matter for international organizations like the EEC and the Council of Europe because of the increasing amount of information which is passed across national borders for processing or storage. The fear is that countries without satisfactory legislation on the subject might become information "havens", analogous to offshore banking centres, remote from the judicial control of the country of origin. The lack of adequate laws in the United Kingdom has become a significant impediment to the growth of the UK data processing industry, causing Sweden not to send data to the United Kingdom for processing.

At the moment there are non-binding guidelines drawn up by the Organization for Economic Development and Cooperation (OECD), and a convention for the protection of individuals with regard to automated data files has been approved but not yet ratified by the Council of Europe. The convention sets out the basic principles of data protection. These include the fair and lawful collection of data, restrictions on the storing of particular data and prohibitions on the storing of sensitive data such as political opinions. The convention also gives anybody about whom data are held the right to obtain information about the nature of the data from the agency officially responsible and to demand that inaccurate data are corrected. What categories of data can be excluded from the scope of the convention and whether dataprotection rights are given to groups of people, associations or companies are left to the discretion of the signatory countries.

The OECD guidelines, which the United Kingdom, Canada and Australia have objected to, are more in line with the views of the United States, particularly the insistence that data should flow across borders unhindered by administrative checks or delays.

Neither of these international codes is yet in operation although many European countries have well established laws on information and data control. The European Parliament's rapporteur, Hellmut Sieglerschmidt (German, Socialist), Fishy proof

This painted goby, the onehundredth species of fish to be found in the River Thames, has been greeted by Thames Water's chairman Geoffrey Edwards as proof that the Thames is the cleanest metropolitan estuary in the world. This follows the "cleanup" campaign of London's sewage system that began in 1960.



concluded that the different national rules are damaging for both the individual and for the data processing industry. In his view, the convention proposed by the Council of Europe leaves too many aspects to the discretion of signatory states. Sieglerschmidt called for a more radical EEC directive. Ideas in this proposal include: the same level of protection in both private and public sectors; the obligation for data holders to notify persons on whom information is held and permit corrections to be made; and the right of the subject of the data to claim for damages against data holders. These proposals were toned down by amendments from Conservatives. Jasper Becker

Science parks

Easy answer?

With cuts in government support hitting UK universities hard, the scramble to set up science parks is on — 15 were launched last year. Few university schemes are actually in operation, and newcomers must look to Trinity College, Cambridge, the first British academic institution to open a science park, for the blueprint.

"Science park" is a vogue term applied to many different schemes. Local authority backed projects, such as that in Warrington, aim to attract high technology industry in the hope of creating more jobs. The universities hope that science parks will earn extra income and provide an environment in which academic scientists and entrepreneurs can put new technology onto the market.

In 1973 Trinity earmarked 140 acres on the outskirts of the town for development. Phase I is now complete and the site houses 23 companies. When Napp Laboratories Ltd, a pharmaceutical company, moves into the new building it is constructing, the number of people working on the site will total 1,000. Trinity is well pleased with its park, collecting around £250,000 a year from lease arrangements, and content at having no equity shares in any of the companies. No companies have moved out and several have expanded their premises. "Every major university should have one", commented John Bradfield, bursar of Trinity.

The park's tenants range from small companies founded by one or two business-minded science graduates to

divisions of multinational companies. LKB Biochrom Ltd, the British subsidiary of a Swedish firm, was attracted to the park because of its proximity to a prestigious university. LKB moved to the park in 1974 and produces analytical instruments used in life science laboratories. According to Mr David Storey, LKB's managing director, the attitude of academic researchers to commerce has undergone "a remarkable change over the past ten years". Many members of the university now act as consultants on either an informal or formal basis, in sharp contrast with the hostility met by IBM twenty years ago when it tried to find premises in Cambridge.

Laser-Scan Laboratories Ltd, which came to the park in 1973, grew directly out of a university department. Founded in 1969 by three researchers from the Cavendish Physics Laboratory, Laser-Scan produces machines, used by the Ordnance Survey and the Ministry of Defence, based on laser scanning technology. Products made by the university engineering department's Microcircuits Group, which is supported by GEC, British Telecom and the Science and Engineering Research Council, will be marketed by a company on the park, Lintech Instruments. Although the park has demonstrated, as one research worker put it, that academics need not "wholly prostitute themselves to become entrepreneurs", it is not yet looked on as part of the university. Its distance from the university prohibits such an intimate involvement.

Cambridge Science Park is an example of a scheme established in response to government pleas in the late 1960s that industry and the universities form closer links. In 1969 a Cambridge committee chaired by Sir Nevill Mott recommended expansion of science-based industry close to the university. Dr Bradfield, who has guided the scheme from its inception, sees science parks as a means of educating academics in the commercial development of their ideas. The Cambridge Science Park has, in this respect, proved successful. But to look to science parks for a significant boost for British high technology, or as a way for universities to replace lost income, is optimistic. High technology companies are notoriously high risk ventures and present conditions are not conducive to overnight success. Jane Wynn

ORRESPONDENCE

US cancer trends

Sir - Your article on the US Surgeon General's report The Health Consequences of Smoking: Cancer 1982 (Nature 4 March, p.4) again draws attention to the importance of smoking as a cause of cancer. The 'remarkable increases in mortality from smoking-related cancers", a widely recognized phenomenon, is usefully reiterated by the Surgeon General. The concurrent decline in the death rate from all non-smoking-related cancers, also undisputed, is reported in the Nature news item. We would like to point out a misinterpretation of the reasons for the decline in cancers not related to smoking which has followed publication of the report.

The Surgeon General cites "improvements in survival rates for some cancers through earlier or better diagnosis and treatment' during the period of steeply increasing smoking-related cancer mortality rates. As had been well publicized, improvements have been made in the treatment of Hodgkin's disease, some childhood leukaemias, testicular cancer, and a few others. In contrast, however improvements in survival of people afflicted with solid tumours, those responsible for the vast majority of cancer deaths, have been small. The improvements in survival, while important, have had little effect on overall mortality from non-smoking-related cancers.

The major contributions to reduced nonrespiratory cancer mortality are the falling mortality rates for stomach and uterine cancers. These declines result not from improved survival, but from declining incidence rates for these cancers1

Stomach cancer rates are now decreasing throughout the developed world. In the United States the trend continues downwards throughout middle age, suggesting that as younger people age, they will have rates lower than those of their parents' generation. Stomach cancer mortality rates in the United States are now among the lowest in the world. Early detection and improved treatment has had little impact on the mortality rates. There has been practically no improvement in survival of stomach cancer patients since 1950.

No single explanation adequately explains the decrease in stomach cancer incidence, but several factors have been suggested as contributors: modern techniques of food preparation and storage, increased consumption of green vegetables, fruits, antioxidants (as food preservatives) and increased milk intake.

The long downward trend in cervical cancer, which began at least 40 years ago, is the chief reason for the large, steady decrease in female non-respiratory cancer death rates over the past half century. The decline began long before the "Pap test" for early detection of cancer of the cervix became widespread. The causes of this substantial improvement are not fully understood, though it appears to have accompanied general improvements in public health and living standards.

The Nature news article cites improvements in survival of prostatic, colorectal and breast cancer patients as contributing to reductions in non-smoking-related cancer mortality. In fact, mortality rates for these three cancers have remained nearly constant since 1950.

While the contemporary declines in

mortality from stomach and uterine cancers appear to be fortuitous concomitants of modern life, additional major reductions in cancer rates can be achieved with present knowledge. The evidence indicting smoking provides the greatest unrealized potential for reducing the impact of cancer

> MICHAEL GOUGH HELLEN GELBAND

Office of Technology Assessment, US Congress, Washington D.C., USA

1. Assessment of Technologies for Determining Cancer Risks from the Environment (Office of Technology Assessment, US Congress, 1981).

Anthrax island

Sir - We would like to deal with a number of points raised by Dr Sterne (Nature 4 February, p.362) regarding our paper on anthrax contamination on Gruinard Island1.

The main object of the 1979 survey of Gruinard Island was to determine whether or not the whole island was grossly contaminated with Bacillus anthracis. We have shown that it is not, but that the organism is found in detectable numbers in a small area.

We believe that Dr Sterne has misunderstood our view about Van Ness's assumption that B. anthracis proliferates in the soil under certain conditions². We stated in our paper that we did not believe such conditions existed on Gruinard Island. The present extent of the contamination was probably due solely to the wartime trials with some slight redistribution of the spores by wind and rain.

We agree with Dr Sterne's statement that he failed to recover B. anthracis from cadavers of cattle buried as little as three months previously because it is known that only spores survive for any length of time. The vegetative bacilli only sporulate in the presence of oxygen3,4, and veterinary officers dealing with suspected cases of anthrax do not normally open the body cavity thus ensuring that oxygen is excluded.

We have shown that if blood is added to contaminated soil from Gruinard Island the spores will germinate and proliferate. The numbers of indigenous soild bacteria did increase as a result of this procedure but the selective medium allowed us to obtain accurate counts of B. anthracis colonies. Dr Sterne rightly states that there is no experimental evidence that B. anthracis does multiply in the soil but what we have tried to show is that the possibility of proliferation in the presence of nutrients does exist and should not be entirely disregarded.

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Improbable stuff

Sir - That the information content of terrestrial biology is truly prodigious¹ seems generally agreed. Hoyle and Wickramasinghe² avoid the problem of assessing repetitiveness and irrelevancy in the DNA (Nature 1 October 1981, p.333) by starting from the fact that 2,000 enzymes are common to all life. Each enzyme has a distinct "backbone" of, say, a dozen amino acids together with a specific active site which catalyses a particular biochemical reaction and is determined by, say, six further amino acids. It is presumed that these common enzymes determine a minimum information content in biological DNA.

Taking the twenty biological amino acids, one can compute the number of different combinations of Boltzmann's "permutability" P of enzyme-like chains: for 2,000 18-link chains P would be $\sim 10^{40,000}$ for 20,000 18-link chains containing the 2,000 specific ones it would be $\sim 10^{-26,000}$, while for 2,000 6-link chains it would be $\sim 10^{-13,000}$. In choosing the number of 6 to characterize the active site, Rout (Nature 18 March 1982, p.192) discounted the "backbone" but more importantly forgot that activities such as energy release from sugars or accurate DNA replication require sequences of particular enzymes catalysing reactions in a specific order.

Weighting the amino acids according to some expected abundances modifies the numbers but an index of the order of 10,000 is inescapable - that is, a probability of 1:1010,000 against finding the present enzyme system in a particular sample at a particular time as a chance arrangement. To demonstrate the immensity of such numbers, note that the product of the number of bacterium-sized drops (10⁻¹³ cm³) in the upper 1 metre of the Earth's surface water and the number of milliseconds in 4 billion years is 10 54 Alternatively, the number of UV photons over 4 eV (capable of breaking and rearranging a chain) arriving through the Earth's history is 10 49. All Earth-like planets in the Galaxy throughout its lifetime would give "only 10 ⁷⁸ or 10 ⁷³

It is probable that some simple selfreplicating systems exist that are viable in restricted environments, if only because present biology can prosper in extreme conditions and utilize a wide variety of exothermic chemical transformations 2. So life might have started from a very simple biology, arising by chance on the primitive Earth. Experimental demonstration of a practical system is not inconceivably remote 4

But why don't geocentric evolutionists (Nature 18 March 1982, p.198) face up to the awkward questions; where are all the simpler intermediate biologies - surely some should have had advantages over our unnecessarily rich biology and have left clear traces? And why should bacteria be adaptive to nonterrestrial environments and in particular be capable of space travel?

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CHARLES DARWIN 1809-1882

The century since Darwin

Charles Darwin was born on 12 February 1809 and died on 19 April 1882. Here John Maynard-Smith reflects upon the 100 years since Darwin's death. A contemporary controversy is discussed in News and Views.

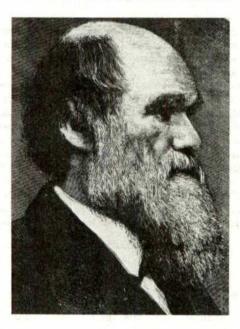
LUDWIG BOLTZMANN once wrote that the nineteenth century would be remembered as the century of Darwin. One hundred years after Darwin's death this judgement still seems perceptive. No other writer had such a profound effect on the way we see ourselves, and no other brought about so great an extension in the range of subjects which we regard as explicable by scientific theory. Here, however, I shall confine myself to his contribution to evolutionary biology, and shall forget that he was the founder of ecology and ethology, and made significant contributions to geology and psychology. It was, after all, his formulation of the theory of evolution by natural selection that was decisive.

In recent years there have been claims in the daily press, on television, and by retired cosmologists - that Darwin may have got it wrong. Some excuse can be found in the fact that Darwin has indeed been criticized by scientists working in a variety of fields - for example palaeontology, taxonomy and embryology. At least one group of scientists has claimed that a new evolutionary paradigm is on the way. However, to see Darwinism as being under serious threat would, I think, be a false perception. The error arises because Darwin's theory is so central to modern biology that any new idea may first be seen (as Mendelian genetics was seen) as being in conflict with Darwinism.

Let me first give a brief history of evolutionary ideas since Darwin. In the Origin of Species, Darwin aimed to establish two things. First, he argued that evolution had in fact happened (that is, that all existing organisms are descended from one or a few simple ancestral forms), and, second, that the main cause of evolutionary change was the natural selection of variations that were in their origin non-adaptive. The main weaknesses of his position were that he had no adequate theory of genetics, and that he could give no satisfactory account of the origin of the variations on which selection would later act. In genetics, Darwin was a Lamarckist. That is, he thought that if organisms acquired characteristics by use and disuse during their lifetimes, this would influence the nature of their offspring. In thinking this, he was sharing an opinion held by almost all his contemporaries.

The first major advance after Darwin was made by August Weismann, who

argued for the independence of the 'germ line' (the cell lineage leading from the fertilized egg to the germ cells, egg and sperm, which form the starting point of the next generation) from the 'soma' (the cell lineage from fertilized egg to adult body). As a boy, I acquired, from reading the preface to Shaw's *Back to Methuselah*, a picture of Weismann as a cruel and ignorant German pedant who cut the tails off mice to see if their offspring had tails. What a ridiculous experiment! Since the



mice did not actively suppress their tails as an adaptation to their environment, no Lamarckist would expect the loss to be inherited. Much later, I discovered that Weismann was not as I had imagined him. His experiment on mice was performed only because, when he first put forward his theory, he was met with the objection that, (as was, it was claimed, well known) if a dog's tail is docked, its children are often tailless — an early use of what J.B.S. Haldane once called Aunt Jobisca's theorem, 'It's a fact the whole world knows'.

Much more interesting are Weismann's reasons for proposing his theory, and its implications for Darwinism. At first sight, his reasons were poor. It is often not the case (for example, in higher plants) that the germ line is a lineage distinct from the soma. Even when it is distinct, the material

and energy it needs are supplied by the soma. In Weismann's day, the experimental evidence for the 'non-inheritance of acquired characters' was weak. Why, then, did he believe it? I think that the clue lies in his remark that, if one were to come across a case of the inheritance of an acquired character, it would be as if one were to send a telegram to China and it arrived translated into Chinese. This is the first use known to me of the information analogy in heredity. Weismann did not accept the inheritance of acquired characters because he could not conceive of a mechanism of 'reverse translation', whereby the hypertrophied muscles of the blacksmith could be translated into genes (he called them 'ids') which could, in the next generation, cause the growth of large muscles.

If Weismann was right, this greatly strenghthened Darwin's theory. Natural selection, instead of being just one of the possible processes leading to evolutionary adaptation, becomes the only process (at least, until the evolution of organisms sufficiently intelligent to learn from their parents).

The next important step was the rediscovery of Mendel's laws at the start of this century, and the formulation of the chromosome theory of heredity. The first impact of Mendelism on evolutionary biology was distinctly odd. The early Mendelians saw themselves as anti-Darwinians; Darwin's banner was held aloft by the biometric school, who concentrated on measuring the correlations between relatives, and who regarded genes as metaphysical entities. The Mendelians saw the 'mutations' they studied as each being the potential starting point of new species, and the continuous variation studied by the biometricians as evolutionarily irrelevant; the biometricians saw mutations as pathological deviants doomed to early elimination by selection, and continuous variation as the stuff of evolution. The argument, led by Bateson on the one side, and Pearson on the other. foreshadowed the current debate between punctuationists and gradualists. It is part of the larger debate between those who see the world as continuous and those who think it proceeds in jerks.

The debate, at least in the form in which it then presented itself, was largely settled by the work of the population geneticists,

NATURE

THURSDAY, APRIL 27, 1882

CHARLES DARWIN

ERY few, even among those who have taken the keenest interest in the progress of the revolution in natural knowledge set afoot by the publication of the "Origin of Species"; and who have watched, not without astonishment, the rapid and complete change which has been effected both inside and outside the boundaries of the scientific world in the attitude of men's minds towards the doctrines which are expounded in that great work, can have been prepared for the extraordinary manifestation of affectionate regard for the man, and of profound reverence for the philosopher, which followed the announcement, on Thursday last, of the death of Mr Darwin.

Not only in these islands, where so many have felt the fascination of personal contact with an intellect which had no superior, and with a character which was even nobler than the intellect; but, in all parts of the civilised world, it would seem that those whose business it is to feel the pulse of nations and to know what interests the masses of mankind, were well aware that thousands of their readers would think the world the poorer for Darwin's death, and would dwell with eager interest upon every incident of his history. In France, in Germany, in Austro-Hungary, in Italy, in the United States, writers of all shades of opinion, for once unanimous, have paid a willing tribute to the worth of our great countryman, ignored in life by the official representatives of the kingdom, but laid in death among his peers in Westminster Abbey by the will of the intelligence of the nation.

It is not for us to allude to the sacred sorrows of the bereaved home at Down; but it is no secret that, outside that domestic group, there are many to whom Mr Darwin's death is a wholly irreparable loss. And this not merely because of his wonderfully genial, simple, and generous nature; his cheerful and animated conversation, and the infinite variety and accuracy of his information; but because the more one knew of him, the more he seemed the incorporated ideal of a man of science. Acute as were his reasoning powers, vast as was his knowledge, marvellous as was his tenacious industry, under physical difficulties which would have converted nine men out of ten into aimless invalids; it was not these qualities, great as they were, which

impressed those who were admitted to his intimacy with involuntary veneration, but a certain intense and almost passionate honesty by which all his thoughts and actions were irradiated, as by a central fire.

It was this rarest and greatest of endowments which kept his vivid imagination and great speculative powers within due bounds; which compelled him to undertake the prodigious labours of original investigation and of reading, upon which his published works are based; which made him accept criticisms and suggestions from any body and every body, not only without impatience, but with expressions of gratitude sometimes almost comically in excess of their value; which led him to allow neither himself nor others to be deceived by phrases, and to spare neither time nor pains in order to obtain clear and distinct ideas upon every topic with which he occupied himself.

One could not converse with Darwin without being reminded of Socrates. There was the same desire to find some one wiser than himself; the same belief in the sovereignty of reason; the same ready humour; the same sympathetic interest in all the ways and works of men. But instead of turning away from the problems of nature as hopelessly insoluble, our modern philosopher devoted his whole life to attacking them in the spirit of Heraclitus and of Democritus, with results which are as the substance of which their speculations were anticipatory shadows.

The due appreciation or even enumeration of these results is neither practicable nor desirable at this moment. There is a time for all things—a time for glorying in our ever-extending conquests over the realm of nature, and a time for mourning over the heroes who have led us to victory.

None have fought better, and none have been more fortunate than Charles Darwin. He found a great truth, trodden under foot, reviled by bigots, and ridiculed by all the world, he lived long enough to see it, chiefly by his own efforts, irrefragably established in science, inseparably incorporated with the common thoughts of men, and only hated and feared by those who would revile, but dare not. What shall a man desire more than this? Once more the image of Socrates rises unbidden, and the noble peroration of the "Apology" rings in our ears as if it were Charles Darwin's farewell:—

"The hour of departure has arrived, and we go our ways — I to die and you to live. Which is the better, God only knows." T.H. HUXLEY

Fisher, Haldane and Wright. Two points were made clear. First, the continuous variation studied by the biometricians could be explained by alternative alleles at many loci, each by itself having a small effect on the phenotype. Second, even rather small differences in fitness between genotypes are sufficient to determine the direction of evolutionary change, despite mutation being mainly in an opposition direction.

The work of the population geneticists prepared the way for the 'modern synthesis' of evolutionary biology, developed in the period 1930–1950 by a group including Dobzhansky, Ford, Julian Huxley, Mayr, Muller, Rensch, Simpson and Stebbins. It is hard in a few sentences to describe what these men did. In effect, they showed that the 'neo-Darwinian' mechanism — natural selection in Mendelian populations — was sufficient to explain the evolutionary process as it could be observed in nature. Dobzhansky, Ford and others measured genetic variability and natural selection in wild populations.

Mayr and Rensch (for animals) and Stebbins (for plants) studied geographical variation within and between species, and discussed how new species might arise. Simpson aruged that the fossil record could best be understood in Darwinian terms. Most research in evolutionary biology since that time has been carried out in the framework of the modern synthesis. Particular efforts have been made in areas which, at least at first sight, seem to be difficult to explain in terms of natural selection, for example, the evolution of social behaviour and of sex and breeding systems.

Since 1950, developments in molecular biology have had a growing influence on the theory of evolution. The 'central dogma' of molecular biology, according to which information can pass from nucleic acid to protein, but not from protein to nucleic acid, provides a molecular explanation for Weismann's principle, thus leaving natural selection as the only agent of adaptation. Important as this is, however, two additional points should be

made. First, even if 'reverse translation' of amino acid sequences into base sequences were possible, this would not provide a general mechanism for Lamarckian inheritance, because most developmental adaptations do not involve the production of new protein sequences. Second, there are good reasons why, even if living organisms have arisen independently many times in the universe, Lamarckian processes should play a minor role in their evolution. Most 'acquired characters' are non-adaptive they are the results of age, injury and disease. Therefore, a hereditary mechanism which transmitted such characters to offspring would work against the evolution of adaptation. Hence the one-way flow of information from nucleic acid to protein may have been a necessary feature of an hereditary mechanism able to support evolution. In physics, the second law of thermo-dynamics asserts that entropy will increase in a closed physical system. In biology, Weismann's principle, together with the principle of natural selection, makes possible the maintenance,

and even the increase, of information in open biological systems.

Molecular biology has had an impact on evolutionary theory in other ways. Protein electrophoresis has provided a way of measuring the genetic variability of populations; its main value may be in enabling us to discover more about the breeding structure of populations. Sequence data on proteins and nucleic acids can be used to work out the phylogenetic relationships of existing organisms. In this context, sequences have the advantage over morphological data in that they provide a means of estimating the number of genetic changes separating two forms. The information we are acquiring about how DNA is arranged in chromosomes may at last give us some insight into the evolutionary significance of chromosome structure. Two questions in particular are being asked. First, does the large-scale arrangement of genes on chromosomes have any significance for development, or is it merely a way of ensuring accurate gene segregation during cell division? Second, does all the DNA in the genome perform some useful function in the survival or reproduction of the organism, or is some part of it 'selfish' or 'parasitic'? The second question raises a set of problems which are logically similar to those which have been debated for some time by students of the evolution of social behaviour, that is, questions about the levels at which selection acts and the differences between what Dawkins has called 'replicators' and 'vehicles'.

There is, however, one area of molecular biology which seems to me to lag behind the rest. This is the study of the evolution of prokaryotes (organisms such as bacteria lacking a proper cell nucleus). The modern synthesis of the 1940s was concerned with eukaryotes (organisms with a cell nucleus, usually sexual and diploid). Its essential achievement was to bring together two previously separate disciplines — the chromosome theory of heredity and the study of natural populations. The same synthesis is now required for the prokaryotes. There is an abundant knowledge of their genetics, but as yet no adequate synthesis of that knowledge with a study of the natural history of bacteria. For example, we have little idea of the significance of conjugation for bacterial populations; it is as if we had no idea of the significance of sexual reproduction for populations of birds or insects. Population thinking has been well developed for fully half a century, but has yet to be adopted by microbiology.

This essay is an abbreviated version of the introduction to Evolution Now, a collection of recent papers on controversial issues in evolution with commentaries by John Maynard-Smith. Evolution Now is being published by Macmillan Press and, in the USA, by W.H. Freeman. It will be available to Nature subscribers as advertised in next week's issue.

THE LATE MR. DARWIN.

The Times. 3 May 1982

At the scientific meeting of the Zoological Society, vesterday evening, before the commencement of the proceedings, the president, Professor Flower said. - The minutes read recall the fact that at our last meeting we were honoured by a communication from Dr. Darwin, probably his last contribution to that science to which he devoted his life-long labours. No one who heard that paper, showing as it did no sign of faltering from that eager interest which he had always manifested in a subject which he had made peculiarly his own, expected that not 24 hours would elapse before those labours would be brought to a close. During the fortnight that has passed the whole world has been moved at the loss it has sustained, and his work and his character have, more than any other theme, filled the minds of thinking people of all countries, classes, creeds, and occupations. We who humbly follow him in cultivating the science he adorned must feel elevated at the sight of the full recognition accorded to his work. The general acceptance of Darwin as one who has exercised a powerful influence upon the whole realm of human thought, the cordial reception of his remains in our magnificent Abbey, among the illustrious men of whom our country is proud, are triumphs in the history of zoology, for it was mainly zoological observation which led to those philosophical speculations which have made his name famous. The nation's grief at his loss has already found eloquent and feeling expression in many quarters; the resources of our language seem to have been exhausted in bearing testimony to his worth. No words that I could find would add anything to what has been so well said by others; and surely here, if an any place in the world, among those who are always occupied with subjects the pursuit of which has been so profoundly modified by his writings, and among many who loved him as a personal friend, nothing is needed but to mention his name to call forth the strongest feelings of admiration for his work and reverence for character. If it is not given to any of us to: emulate him in brilliancy of scientific induction, or to light upon discoveries that will change the current of human ideas, we can at least endeavour to follow the example he has set us of patient perseverance in observation, scrupulous accuracy of statement, deference for the opinions and feelings of others, candour towards opponents, and of that invariable modesty and gentleness of demeanour which shed such a charm around his public as well as his private life.

LATEST INTELLIGENCE

(FROM OUR CORRESPONDENTS.)

(BY TELEGRAPH.)

FRANCE.
PARIS, MONDAY, APRIL 24 Mr. Darwin has to be added to the long list of eminent men, not excluding Catholic prelates, whom the Univers has denounced or deemed. Its wrath has been excited by the praises of the Radical Justice. The notices in the French Press, by the way, have mostly been very

meagre. It thus winds up two columns of sarcasm:

"When hypotheses tend to nothing less than the destruction of faith, the shurring out of God from the heart of man, and the diffusion of the fifthy leprosy of Materialism, the savant who invents and propagates either a criminal or a fool. Viola ce que nous avons à dire du

The Times, 25 April

TO THE EDITOR OF THE TIMES

Sir,-May I beg a corner for my feeble testimony to the marvellous persevering endurance in the cause of science of that great naturalist, my old and lost friend, Mr. Charles Darwin, whose remains are so very justly to be honoured with a resting-place in Westminster Abbey

Perhaps no can better testify to his early and most trying labours than myself. We worked together for several years at the same table in the poop cabin of the Beagle during her celebrated voyage, he with his microscope and myself at the charts. It was often a very lively end of the little craft, and distressingly so to my old friend, who suffered greatly from sca-sischess. After, perhaps, an hour's work he would say to me.
"Old fellow, I must take the horizontal for it," that being the best relief
position from ship motion; a stretch out on one side of the table for ome time would enable him to resume his labours for awhile, when be

It was distressing to witness this early sacrifice of Mr. Darwin's health, who ever afterwards seriously felt the ill-effects of the Beagle's

J. LORT STOKES, Admiral. Scotchwell, Pembrokeshire, April 25. Lam, Sir. yours truly

The Times, 27 April

CHARLES ROBERT DARWIN

Exactly a year to a day has separated the deaths of two of the most powerful men of this century, some have said of any century; and those who care for the task will find some very curious analogies between the progress and the ultimate results of the work of the two men, totally different as were the spheres in which they exercised their remarkable powers. On April 19, 1881, all the civilized world held its breath at the new of the death of Lord Beaconsfield; not less must be the effect upon the most civilized part of the civilized world when the announcement of the death of Charles Darwin flashes over the face of that earth whose secrets he has done more than any other to reveal. All who knew anything of Mr. Darwin know that, massive as he seemed, it was only by the greatest care and the simplest habits that he was able to maintain a moderate amount of health and strength. Mr. Darwin had been suffering for some time past from weakness of the heart, but had continued to do a slight amount of experimental work up to the last. He was taken ill on the night of Tuesday last, wher he had an attack of pain in the chest with faintness and nausea. The latter lasted with more or less intermission during Wednesday and culiminated in his death, which took place a about 4 o'clock on Wednesday afternoon. He remain conscious to within a quarter of an hour of his death. His wife and several of his children were present at the closing scene During his illness he had been attended by Dr. Norman Moore, Dr. Andrew Clarke, Dr. Moxon, and Dr. Alfrey, of St. Mary Cray.

Fifteen volumes lie before us and nearly as many memoir large and small, the product of 45 years' work - a produc hich, in quantity, would do credit to the most robust constitution. But when we consider Mr. Darwin's always feeble health and his deliberately slow method of work, never hasting but rarely resting, the result seems marvellous. But wonderful as this is under the circumstances, it is not by mere quantity that Mr. Darwin's work will be judged: the quantity is of chief importance in respect of the multifarious channels through which his influence has spread.

The Times, 21 April

NEWS AND VIEWS

Methylation and gene control

from Gary Felsenfeld and James McGhee

ARE changes in DNA methylation patterns a cause or an effect of eukaryotic gene expression? There are already many examples of an inverse correlation between the level of methylation in the neighbourhood of a gene and the transcriptional activity of that gene. It is more difficult to prove that the extent of methylation is in itself sufficient to determine gene activity.

Several recent studies tackle this problem by making use of the inability of bacterial restriction endonucleases to cleave DNA restriction sites that have been methylated. In eukaryotes, the site of methylation is almost invariably the 5' position of cytosine and, in animals, the cytosine residue is usually contained in the sequence CpG. The restriction endonucleases HpaII and MspI, which can cut the sequence CCGG, have therefore proved useful tools in these studies1. Methylation renders the sequence resistant to the Hpall endonuclease, but not to Mspl, and with the use of gel electrophoresis and Southern blotting, the pattern of methylation at those CpG sites which are embedded in a CCGG sequence can be determined in some detail.

It has been shown with these (and other) restriction enzymes that certain CpG sites in the vicinity of a wide variety of genes are undermethylated in a tissue in which the gene is expressed compared with a tissue in which the gene is inactive (reviewed in ref. 2). Unfortunately, only a fraction of methylation sites are also restriction sites and can be detected in this way. The pattern of variation in methylation is complex, and usually does not involve complete demethylation of extensive regions of the DNA, although the pattern tends to be stably inherited within a given cell line. This is presumably because the enzyme bringing about methylation acts much more efficiently on hemimethylated sites than on unmethylated ones, and therefore methylates the daughter strand of a newly replicated duplex wherever the parent strand is methylated.

To show that DNA methylation directly affects gene expression requires an experimental method of altering methylation levels. Recently the *Hpall* methylase has been used to introduce methyl groups into defined DNA sequences. In early studies^{3,4}, the herpes thymidine kinase (tk) gene was methylated in vitro and used to transform mouse LtK-

cells. Methylation decreased the transformation efficiency. and transformants selected for tk expression carried tk sequences that were partially demethylated relative to the input DNA. Though these results are compatible with an inhibitory effect of methylation on tk expression, their interpretation is complicated by the possible effects of methylation on the transformation process itself. Such effects cannot be measured because apparently only cells carrying at least partially demethylated tk sequences survive.

A new experiment by Stein et al. 5 solves the problem by using separate genes for selection and for methylation. Mouse Ltkaprt- cells were transformed with herpes tk DNA, and in addition co-transformed with hamster aprt (adenine phosphoribosyl transferase) DNA. The aprt gene was introduced either in the unmethylated form or after methylation in vitro by Hpall methylase. Clones selected for tk+ activity were then assayed for expression of the cotransformed but unselected aprt gene. (It has been shown earlier6 that methylation patterns of transforming sequences not subject to selection pressure are stable over many generations.) Clones transformed with unmethylated aprt sequences were found to express the aprt gene. Clones transformed with methylated aprt neither transcribed nor expressed the gene, even though the methylated sequences could be detected within the transformed cells by Southern blotting, and appeared to be incorporated into the genome with an efficiency similar to that for unmethylated aprt sequences. When the clones carrying methylated aprt were subjected to conditions selecting for the aprt+ phenotype, reversion was found to be associated with undermethylation of the aprt sequences. The authors conclude that the observed effect of methylation on aprt activity arises from a direct influence of methylation on gene expresion.

The connection between DNA methylation and gene expression is supported by studies of mouse retroviruses. A number of workers had previously shown that the inactive, gene-

Gary Felsenfeld and James McGhee are in the Laboratory of Molecular Biology, NIADDK National Institutes of Health, Bethesda, MD 20205. tically acquired proviral sequences are heavily methylated whereas the active, somatically acquired copies are undermethylated. In a recent study, Stuhlmann et al.⁷ used transfection of cell cultures by purified mouse DNA to show that the nonmethylated proviral sequences were infectious whereas the methylated sequences were not. Furthermore, a copy of the endogenous inactive provirus was cloned in bacteria and the cloned DNA (now unmethylated at CpG sites) was found to be highly infectious⁸.

A possible objection to DNA transfection and transformation studies is that cytosine methylation could direct the site of DNA integration. For example, methylated DNA could integrate into methylated regions of the chromosome, which are inactive for other reasons. Perhaps this objection could be overcome by more detailed studied of flanking regions or by transformation with methylated and unmethylated sequences ligated together in the same molecule. In any case, a methyl-directed integration model cannot explain the results of Vardimon et al.9. A region of adenovirus coding for the Ad2-specific DNA binding protein was cloned and the DNA, either left unmethylated or methylated in vitro with the Hpall methylase, was injected into Xenopus oocytes. The results were striking: unmethylated DNA produced adenovirus-specific RNA, whereas the methylated DNA did not.

Levels of methylation can also be deliberately altered by the use of the nucleoside analogue 5-azacytidine (5-azaC), which inhibits DNA methylation in vivo. Jones and Taylor¹⁰ have shown that 5-azaC can induce differentiation in cultured mouse embryo cells, and that at the same time and dosage the cellular DNA becomes demethylated. Three other cytidine analogues behave similarly, whereas two more that do not induce differentiation do not affect methylation. Two recent studies have extended this approach to an analysis of the state of individual genes after cell growth in 5-azaC. Groudine et al. 11 report that 5-azaC induces expression of an endogenous avian retrovirus, and that this expression is associated with both DNA demethylation and increased nuclease sensitivity of the viral sequences in chromatin. Compere and Palmiter12 have studied the effect of 5-azaC on the

expression of the metallothionein gene in a mouse thymoma cell line in which the gene is normally uninducible. After exposure to 5-azaC, sites on the gene are unmethylated and the gene becomes inducible. Finally, 5-azaC has been used to enhance the reactivation of the inactivated human X chromosome in mouse-human cell hybrids13, an effect consistent with the proposal that DNA methylation is the basis of X chromosome inactivation in female mammals13,14.

The mechanism by which 5-azaC causes demethylation is unclear. The analogue apparently must be incorporated into DNA to inhibit methylation^{10,12}, but it cannot function merely by virtue of a lack of a methylatable atom in the 5 position, since the extent of demethylation far exceeds the amount of 5-azaC incorporation. Furthermore, not all genes can be activated by non-lethal concentrations of 5-azaC^{5,15}, perhaps because activation of some genes requires more extensive demethylation than can be achieved with non-toxic levels of 5-azaC. There is evidence that methylase can act as a processive enzyme, so that a single 5-azaC in a daughter DNA strand could affect the methylation of cytosines downstream on that strand. If binding of methylase in vivo were coupled to replication, the effect of 5-azaC on gene expression could be a complicated function of the distance between the methylatable control region of the gene and the nearest replication origin.

Although methylation levels are clearly correlated with expression in the cases discussed above, there are some genes, even in organisms with a high general level of DNA methylation, that do not display such obvious correlations. For example, the $\alpha(2)I$ collagen gene of chicken has a pattern of HpaII site methylation that is invariant (with some sites completely unmethylated) in five cell types that have been studied, whether or not the cell type synthesizes detectable amounts of collagen16. Another anomaly has been reported in the case of X chromosomes from normal human cells, which in contrast to X chromosomes from mousehuman cell hybrids reveal no simple correlation between chromosome inactivation and the pattern of methylation at Hpall sites. Although these may be examples of situations in which control of expression is not coupled to altered levels of methylation, it is also possible that the crucial methylation sites are not Hpall sites.

In any case, these results are not inconsistent with models in which absence of methylation at specific residues is necessary for gene expression. Analysis of existing data suggests that the methylation sites critical to a given gene's control processes may be a small fraction of those sites present in that DNA region. The effect of methylating these critical sites presumably is be to alter the way in which proteins interact with the region, either by

modifying local interactions with the active sites of sequence-specific proteins, or by longer-range effects on DNA structure. In the synthetic polynucleotide poly(dC-dG). poly(dC-dG), which consists entirely of CpG repeats, methylation of the cytosine residues causes the polymer to adopt the left-handed Z DNA conformation under physiological salt conditions¹⁷. Furthermore, the Z form has so far proven incapable of forming nucleosomes¹⁸. Such results lead quite naturally to the idea that methylation in vivo may have a major effect on DNA and chromatin structure, but one should not try to be too specific, at this stage, concerning the nature of such effects. There is, for example, at the 5' end of the chicken adult β globin gene a region 200 base pairs long, which contains about six times the genomic frequency of CpG sites, and which is exposed, apparently nucleosome free and reduced in methylation when the globin gene is active19. Examination of the nucleotide sequence does not, however, reveal any extended sequence of alternating purines and pyrimidines of the kind required for Z DNA formation, so that the effects (if any) of methylation on the structure of this region are likely to be more subtle.

Although it now appears that DNA methylation plays an important role in gene expression during development, it should be recalled that some organisms (notably insects) manage quite well without any extensive methylation. The mechanisms for marking expressed genes in such organisms, like the mechanism that marks genes for demethylation in the organisms discussed above, remains unknown.

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Vitamin A, limb patterns and the search for the positional code

from Jonathan Cooke

By around 1940 such figures as Boveri, Harrison, Child and Spemann had propounded, in essence, all the rival scientific theories that we have subsequently lived with concerning animal morphogenesis, with its reliable and typical spatial patterns of cellular differentiation. There has followed a wealth of phenomenological work fleshing out one or another of these theories, and resulting in refinement or renaming of ideas according to the allegiances of the workers concerned.

But this has been accompanied by so little progress towards real knowledge of the biological machinery of development that some scientists see the efforts of the last forty years as taking place in a world like that of Herman Hesse's allegorical novel 'The Glass Bead Game', where compulsive scholars are forever playing a purely internal and, by prearrangement, interminable game with the pieces of theories. A sour-minded view maybe, but the sad fact is that the various conjectures about morphogenetic mechanisms have swung in and out of fashion over the years in a way that would be more appropriately explained by microsociologists or social historians of science than by the scientists themselves.

As an addicted insider I report the embryological glass bead game to be still alive and compulsively enjoyable, but can nevertheless see the present force of argument for a swing towards a more reductionist, biochemical approach to promote real progress. An opportunity for just this may be presented by two exciting new reports that compounds of the vitamin A family exert systematic effects upon the anatomical patterns of developing and regenerating limbs. The effects are separable from uninformative toxicity, and involve formation of elements of the familiar patterns that are in themselves histologically normal, but in quite abnormal spatial configurations.

In one case Maden1 shows that regeneration from the amputated limb stump of an amphibian is disturbed when its early phases are delayed by the presence of retinoic acid or its derivatives in the bathing medium. The terminal population of locally de-differentiated cells or

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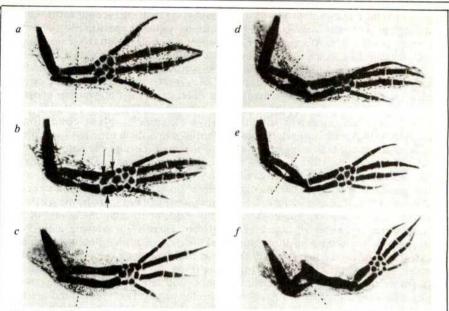
blastema, from which the regenerate develops, normally differentiates to replace just that part of the base-to-tip sequence that was removed by amputation. In Maden's experimental cases, it reiterates an inappropriately large part of this baseto-tip sequence, so that the limb finally regenerated has internal pattern repetition in the long axis. The precise level in the pattern from which the blastema cells now re-commence differentiation, and thus the extent of the final repetition, depends largely on the level of amputation in the original limb and the length of exposure to the vitamin over several days, rather than being a simple expression of the chemical concentration of the agent. Neither delay in initiation of regeneration due to other agencies nor, indeed, any other known manipulation can effect such 'proximalisation' of the positional values recorded in these blastema cells.

In Maden's words, these results "all contravene the rule of distal transformation", a rule that was coined to express the phenomenon that biological patterns seem characterized by an 'uphillto-downhill' polarity, such that regeneration by cells at a cut edge will normally proceed only downhill. The fact that a novel experimental circumstance, and especially a biochemical insult, can by-pass such 'rules' should not, however, surprise us. Organisms consist of evolved arrangements whereby normal processes and results have been assured given normal surrounding circumstances, whereas the pre-biotic world, so far as we know, does not. Thus while Newton might well stir momentarily should two asteroids be observed to jink away from each other as they passed in their orbits, no one coining a 'rule' or 'law' as part of a theory for biological process expects it to be proof against all future insults.

In the other case, Tickle et al. (Nature, in the press) show that early development of the bird limb bud is disturbed by implantation of a local reservoir of retinoic acid in an anterior position within it. But here, the systematic mirror duplications in the antero-posterior axis of pattern, that is, the sequence of digits that are produced, are similar to the result of implanting a particular piece of indigenous tissue — the normally posteriorly situated zone of polarizing activity — to a similar anterior position.

Even a speculative comparison of the vitamin A concentrations experienced by the reacting cells in the two systems is difficult, but in the bird experiments², the local concentration near the source, and perhaps in the entire limb, may be an order of magnitude higher than the upper end of the ambient concentration range in the regeneration study¹. The length of time for which it is present in the limb buds, in relation to the days-long exposure of the blastemas, is unknown (or at least not stated).

It is, at first, astounding that separate



The limbs shown above regenerated after amputation through the mid-radius and ulna (broken line). The distal radius, and ulna, carpals and digits have all been replaced. (a) A control limb which regenerated in pure tap water (b-e) Limbs treated with 15IU ml⁻¹ retinol palmitate for 12 days. In each case pattern elements were added; (b) contains extra carpal-like cartilage; (c) an abnormal length of radius and ulna; (d) a complete extra radius and ulna and (e) duplication as far back as the distal end of the humerus and including a perfect elbowjoint. In (f) a complete limb has been produced, distal to the amputation plane, by exposure to a higher concentration of retinol palonitate (15 days in 75 IUml⁻¹).

Increasing the concentration or time of exposure to retinol palmitate causes the level from which the limb was repeated to become more and more proximal until finally a complete limb is regenerated from the amputation plane. When the position of the amputation plane is varied it is found that higher levels of retinol palmitate are needed to regenerate complete limbs from more distal positions; only the highest concentration at the longest time produced regeneration of a complete limb from the carpal level.

dimensions of the anatomical pattern are exclusively affected in each system, when they are, after all, homologous episodes of pattern formation in related organisms. The entire enterprises of comparative anatomy and causal embryology can, however, be rescued if one chooses to deduce, from other data available, that pattern axes in the present experiments are affected according to whether or not, in each system, certain spatial interactions are possible along them (that is, whether they are in what embryologists refer to as the labile or the mosaic state) at the time of exposure.

Unpublished work at the National Institute of Medical Research suggests that ambient exposure, rather than localised sources, in the chick limb, affects the antero-posterior pattern axis, which is the labile one at the time, but does not cause duplications. So one very abstract scheme would be that proximal and posterior boundaries of the pattern represent 'source' or 'origin' states, and that excess vitamin drives cells progressively and artificially towards these regardless of the state previously dictated by their position. During limb outgrowth, the proximo-distal sequence of pattern contributions evolves locally in the cell population of the tip by means of time itself, time which can be 'set back' by means of simple exposure to the compound. In anterior-posterior pattern formation in the chick limb, by contrast,

polarity has normally to be maintained across a zone of tissue one millimetre wide where the cells are still labile. This is accomplished by prolonged signalling, from a localized source, the zone of polarizing activity. Hence the requirement for a localized reservoir of the experimental agent to provoke a new boundary elsewhere and thus duplication.

But almost nothing can be deduced from the present observations simply by plugging them into current detailed but abstract models, involving such entities as separable cell positional values and 'interpretation' processes, pre-patterns, progress zones, serial inductions and so on (see for example, refs 3-5). All such schemes are, at best, simply consistent with, rather than positively dictated, by the phenomena they have sought to explain, and with the hindsight of final understanding they might even come to be seen as having impeded, rather than helped, attempts to understand effects such as that of retinoic acid.

Anyone who would make sense of the new phenomena had better be prepared to be involved with protein gels, fractionation columns and membrane preparations, for surely the genuine excitment is in the promise they hold out for biochemical investigation of pattern control in appropriate experimental material, via our imperfect knowledge of the loci at which vitamin A inserts itself into the metabolic

functions of the cell. One can only hope for sustained effort rewarded by good luck in any such assisted guessing games, for it would be sad if the present results fared no better than the 'lithium phenomenon'.

Lithium is the best known of a small. chemically diverse group of simple substances, long ago discovered to have systematic distorting effects, this time on primary proportioning of the whole body pattern at its first determination, in ways which are 'homologous' in a wide variety of embryo types. The effects are of the same order of specificity as the present ones. They must be telling us something about the biochemistry or cell biology of spatial organization^{6,7}, but no-one has ever discovered what. Lithium ions, substituting for, and thus distorting, the role of sodium as they presumably do, may affect so many membrane and other functions that the domain to be searched for the relevant effect is very great. We might hope for more success with retinoic acid with its more restricted list of functions. Mediation in the glycosylation of proteins, effects upon gap junction formation and upon the balance of secreted matrix proteins have been mentioned in the publications. These molecular assemblies have each been previously suggested as candidates for involvement in the setting up or maintenance of tissue positional codes.

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T cells immortalized?

from Nigel Williams

SUCCESSFULLY fuse an antibodyproducing B cell with a myeloma cell and there is a good chance that the resulting hybridoma will provide a continuous supply of monoclonal antibodies. Can similar techniques be applied to T cells, the other major population of lymphocytes within the immune system, whose function is much less well understood? The answer that emerged from a recent meeting in Basel* is that in many cases it can. T cells are a functionally heterogeneous population of cells whose antigen-specific surface or soluble receptor molecules remain a puzzle, so the production of functional T-cell hybridomas to provide a monoclonal source is clearly of great interest. How these molecules relate to antibodies and how many different molecules are produced are two major questions to which the production of hybridomas has been addressed.

The function of one major population of T cells is to help B cells produce antibodies. On stimulation by antigen presented by macrophages bearing self molecules by the major histocompatability complex (MHC), T cells can release molecules which help B cells produce antibodies against the same antigen. However, from cloning such T cells it is clear that on stimulation they can also release other factors. Interleukin-2, also known as T-cell growth factor (TCGF), is the best characterized and can stimulate the growth of resting T cells. Stimulated helper T cells can also provide bystander help, that is, they can stimulate antibody production by B cells regardless of their specificity. How this function is achieved is not understood.

M. Schreier (Basel Institute for Immunology) has produced clones of mouse helper T cells generated against egg albumin (EA). While all the clones could provide all these functions, the amount of help for antibody responses against EA and TCGF production varied greatly between different clones and neither of these functions correlated with the amount of bystander help. He has produced hybridomas from these cloned T cells which also preferentially expressed different functions. Although it is not yet clear how far these differences in helper function result from quantitative differences in the production of similar molecules, and how far from differences in the molecules produced, stable factorproducing hybridoma clones should eventually help resolve these questions.

Many of the characteristics of mouse helper T cells have been confirmed in human ones. E. de Freitas (Wistar Institute, Philadelphia) has produced hybridomas between human helper T cells generated against tetanus toxoid and a human T-lymphoma cell line which produce TCGF and also help B cells produce antibodies against tetanus toxoid. As in mice, the expression of these two functions varied in different hybridoma clones. It is not known whether these cells can also produce bystander help but if they do, then the exciting possibility is raised that human antibodies could readily be produced in vitro.

A second population of T cells functions

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of Nature.

to suppress antibody production. Hybridomas have provided a monoclonal source of the molecules involved. Although, like antibodies, these molecules carry an antigen-binding component, some also appear to carry determinants encoded by the I region of the MHC. However it is not clear whether antigen binding and I-region determinants are found on a single polypeptide chain.

M. Taniguchi (Chiba University) described recent work on hybridoma clones producing a suppressor factor specific for the antigen keyhole limpet haemocyanin (KLH), expressing I-J-region determinants. This could be extracted from the hybridoma cells or obtained in the ascites from hybridoma-bearing mice as a secreted factor. By use of immunoabsorbent columns he showed that the activity of the extracted factor could be reconstituted by mixing equal proportions of eluates from an anti-I-J column and a KLH column. If the secreted factor was first subjected to reducing conditions, similar reconstitution of activity could be obtained by mixing eluates from these two columns. This evidence for a two-chain factor is supported by experiments in which mRNA extracted from the hybridomas was injected into Xenopus oocytes where it was translated. The mixture of equal quantities of two translocation products showed strong suppressor activity with KLH specificity, while either product alone was without effect.

D. Webb (Roche Institute of Molecular Biology, Nutley) has also produced T-suppressor cell hybridomas, but specific for the synthetic polypeptide GAT, in two different strains of mice. One of these strains can respond and produce antibody following challenge with the antigen, whereas the other (non-responder) strain is unable to produce anti-GAT antibody. However specific suppressor T-cell releasing factors can be found in both mice. These have been highly purified from the hybridomas and one has recently been reported (Proc. natn. Acad. Sci. U.S.A. 97, 1254; 1982). (Such a feat demonstrates the importance of a monoclonal source to obtain 2 mg of factor, 10 litres of supernatant were required. Slightly better yields were obtained from the extracted factor.)

It has been shown that these molecules have an antigen-binding region and carry I-J-region-encoded determinants. However it seems that the factor from the non-responder mice is a single polypeptide while in the responder strain there are two different factors: one a single-chain and the other a two-chain polypeptide. Cloned cDNA encoding these molecules has now been produced and should provide further illumination.

In another system where suppressor T cells control the contact sensitivity response to the hapten nitrophenol, M. Dorf (Harvard University) described a series of three interacting suppressor cell

^{*}A meeting on T-cell hybridomas was organized by and held at the Basel Institute for Immunology, 27-29 January 1982.

types which release factors, from which hybridomas have been produced. Again such factors carry *I-J*-region determinants and have antigen-binding (or in the case of the second type TsF_2 , idiotype-binding) specificity. Preliminary experiments from immunoabsorbant columns were described which showed that the activity of TsF_2 could be reconstituted from equal amounts of eluates from an anti-idiotype column and anti-*I-J*-column following treatment of the factor under reducing conditions. This suggests that this factor in the series consists of two chains linked by a disulphide bond.

As T-cell hybridomas provide a monoclonal source of an increasing array of molecules it is crucial that their function is clearly established. Stressed by J. Miller, this point is underlined by a recent report by D. Capra (Pacifico & Capra J. exp. Med. 152, 1289; 1980). He originally claimed (Clark & Capra J. exp. Med. 155, 611; 1982) to have produced a hybridoma clone releasing an antigen-specific suppressor factor bearing I-region-encoded determinants. However the factor was not functionally tested and it now appears that the molecule can be recovered from lysates of other unrelated cells, although it is not yet known if this molecule carries I-region determinants.

The function of the T-cell molecules is one of the central questions facing immunologists; the other is that of their relationship to antibodies. The antigen-binding portion of an antibody molecule is encoded by a group of variable region genes. The rest of the molecule is encoded by constant region genes (those for the heavy chain determine the class of antibody). It is not yet clear whether the antigen-binding region of the T-cell molecules is encoded by antibody variable region genes. Some seem to bear variable region determinants while others do not.

Possible evidence for constant region determinants was described by E. Culbert (University College London). He has produced hybridomas from antigenspecific helper and suppressor T cells. Rabbit antisera raised against these factors appear to be able to descriminate between helper and suppressor hybridomas regardless of antigen specificity. Taniguchi has antisera that seem to detect a constant region determinant on the antigen-binding chain of his two-chain suppressor molecule. These seem to be encoded by genes close to the heavy chain constant region genes of antibody, as the antisera, raised in antibody heavy chain congenic mice, can detect determinants selectively expressed on T cells.

It is clear that the application of hybridoma technology is helping to provide an immortalized source of T-cell molecules. Although several different antigen-specific molecules have now been produced by these means, their precise relationship to antibodies remains tantalizingly obscure.

How cells in distress use SOS

from S.G. Sedgwick and G.T. Yarranton

TREATMENT of eukarvotic and prokarvotic cells with DNA damaging agents induces a diverse and somewhat bewildering variety of cellular responses. One interpretation is that cells in general coordinately control operons which are expressed only in response to DNA damage. A model system of DNA damage-induced gene expression is the SOS response of Escherichia coli. This response entails de-repression of at least 12 different operons. The response alters DNA repair capability, mutagenesis, restriction modification and cell division control, and leads to expression of several different din genes, identified only by their inducibility by DNA damage. In addition, certain prophages, such as \(\lambda\), are de-repressed and enter a cycle of lytic growth. It has been supposed that the phages have evolved to take advantage of the SOS response so that they can abandon a host cell whose viability is uncertain.

Although the genes which are switched on participate in several metabolically distinct pathways, they appear to be part of a single regulatory unit, the SOS regulon, whose expression is controlled by the *lexA* and *recA* genes. The idea that lexA protein is a multisite repressor switching off transcription of the *recA* and several other genes, including its own, has recently gained direct support.

Little et al.2 and Brent and Ptashne3 have purified lexA protein and examined its binding to purified control sequences of the lexA and recA genes. Both DNAs contained 20 base pair palindromic sequences, or SOS boxes, which were protected by lexA protein against nuclease and methylation treatments. The recA gene has a single box, whilst the lexA gene has a tandem pair. Significantly, all three binding sites show considerable homology with one another^{2,4} and with sequences preceeding two other genes, uvrA and uvrB5,6,6A, which are also part of the SOS regulon. Repressor activity in vitro was demonstrated directly by specific inhibition of transcription from both lexA and recA promoters by purified lexA protein. Further evidence of multigenic repression by lexA protein was provided by analogous experiments in which lexA protein blocked in vitro transcription from cloned uvrB6A and dinA and dinB genes7. Thus, during normal growth, the genes of the SOS regulon would be repressed by lexA protein which would be maintained at a constant level by the autoregulatory repression which lexA protein exerts on its own synthesis.

Predictions that induction of the SOS

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regulon is caused by proteolytic inactivation of lexA protein by recA protein have also been confirmed. Little et al. 2 showed in vitro that homogeneous recA protein cleaves purified lexA protein into two fragments that cannot repress transcription from the recA promoter. They used a mutant form of recA protein that has stronger than normal proteolytic activity, but similar proteolytic action by wild-type recA protein is thought to be activated following damaging treatments by single-stranded DNA or DNA degradation products. The proteolysis of lexA protein therefore closely resembles the \(\lambda\) repressor cleavage reaction described by J.W. Roberts and his colleagues8. Indeed, the cleavage site is in a region with a similar composition to that of λ repressor⁹ and may thus provide a molecular example of convergent evolution — λ repressor has evolved to mimic lexA protein as a substrate of proteolysis by recA protein.

Clues to how finer regulation of the SOS response occurs are also appearing. First, recA cleaves lexA protein at least ten times more efficiently than it does λ repressor. This may favour survival of λ by ensuring that lysogenic induction does not occur until the levels of DNA damage exceed the capacity of the inducible repair systems9. Second, the recA SOS box binds lexA protein about ten times more strongly than do the pair of boxes preceeding the lexA gene³. Thus, various SOS operons with different variations of the SOS box sequence may be derepressed after different amounts of lexA protein have been inactivated. The reason why there are two SOS boxes at the lexA locus is not clear, though Brent and Ptashne suggest that their lower affinity would permit de novo lexA protein synthesis during induction. As a result, the period in which lexA levels fall might be extended, permitting finer discrimination for switching on part, but not all, of the regulon. Lower repressor affinity of the lexA operator might be expected on theoretical grounds since the gene must presumably synthesize sufficient product to ensure repression of the rest of the regulon before it switches off its own expression.

Third, the relative position of SOS promoter and operator sequences vary^{2,3}. However, the importance of this feature is unclear and the relative binding constants of promoters at different sites must be determined before the complete kinetic analysis of gene expression during induction can be made.

A second refinement of the basic SOS model must be introduced to account for at least two genes which can be regulated independently, or as part of the SOS regulon. The uvrB gene, which codes for a

subunit of the UV endonuclease, was found by Fogliano and Schendel¹⁰ to be under lexA control. Subsequent sequence analysis of the uvrB control region5,6A revealed adjacent non-overlapping promoter sequences. The P2 promoter, furthest from the initiation codon, spans an SOS box⁵ protected from in vitro nuclease digestion by lex A protein, suggesting that this is the site where lexA repression occurs. Control of expression from P1 is as yet unresolved, although van den Berg et al. suggest that the uvrC protein may be involved. Interestingly, Sancar et al. 11 have identified and purified the uvrC protein and shown that it binds strongly to DNA. The third promoter, P3, located 341 base pairs before the structural gene, produces an in vitro transcript terminating in the region of lexA binding site. It is unclear whether P3 has a role in in vivo uvrB gene expression^{6A}. Identification of a second gene, himA, with dual control has recently been described by Miller et al. 12. The himA gene product is needed for site-specific recombination, best studied by integration of phage & DNA into the E. coli chromosome. λ integration accomplished by the combined action of λ int protein and host integration factor. himA's function is to encode one subunit of the host integration factor, the other subunit is probably specified by the himD gene. himA was found to resemble other genes in the SOS regulon in being induced by UV and mitomycin C provided the recA and lexA genes were functioning normally. However, himA and himD are also subject to an alternative control mechanism, since both genes are expressed at high levels when either one of their gene products is inactive. This indicates that himA and himD may jointly repress their own syntheses as well as forming host integration factor. Until the controlling regions of these genes have been sequenced, it is uncertain whether or not lexA, himA and himD proteins act at the same or different sites, and whether himD is also regulated by lexA.

Remarkably, the role of himA in lysogenization is not limited to providing a subunit of the host integration factor. Earlier work by Miller13 showed that humA

was also needed for efficient transcription from the λ promoters P_E and P_I , which direct synthesis of λ repressor and integrase. Miller suggested that himA possibly repressed the bacterial hfl gene whose product decreases activation of P_E and P₁ by \(\lambda \text{III}\) protein. Thus, \(\lambda \text{im} A\) also aids lysogenization by boosting synthesis of λ repressor and integrase. Int protein and host integration factor are also required for prophage excision during lysogenic induction, showing again how \(\lambda\) exploits

the SOS system to leave a host which might be dving.

The existence of at least two genes with dual regulatory mechanisms raises the possibility of a general scheme where many components of the SOS regulon may be capable of independent expression for 'routine' metabolism. Superimposition of a second SOS regulatory system would provide a means of coordinating diverse and unrelated metabolic pathways to aid cellular survival.

A Soviet view of the venusian surface

from Lionel Wilson

A highlight of this year's Lunar and Planetary Science Conference* was the session devoted to Venus, at the close of which Soviet scientists presented photographic panoramas and chemical analyses of the Venus surface obtained by the recent soft-landers Venera 13 and 14. The resolution of the new images is far better than those from earlier Soviet probes and the chemical data provide the first opportunity to make 'direct inferences about the internal geochemistry of Venus.

Studies of the Solar System over the last decade have shown that the other silicate planets - Mercury, Venus and Mars display a variety of surface features produced by processes identifiable on Earth; but no planet yet examined has a crust dominated by the kinds of largescale, plate tectonic processes which typify the Earth's outer layers. Until recently, however, the surface of Venus, the only planet truly comparable with the Earth in terms of size and mass, remained hidden beneath the dense, cloud-rich, CO₂ atmosphere. In 1975, the soft-landing Soviet spacecraft Veneras 9 and 10 provided low-resolution pictures of two parts of the surface, showing boulderstrewn terrains; but, although there was evidence of vesicular rocks1 these pictures only gave hints at the structures visible and the processes which might have formed

Attention switched to the large-scale structure of the crust of Venus when the US Pioneer Orbiter reached the planet late in 1978 and began to map the surface topography by radar. A consensus of opinion2 held that Earth-style plate tectonic processes were at best poorly developed on Venus and that heat was released from the interior at a series of localized mantle hotspots above which active volcanoes opinion, held that Earth-style plate tectonic crustal uplifts, the most prominent being the Beta Regio region. The Pioneer measurements, coupled with Earth-based radar data on surface roughness and dielectric constant, showed that the southern part of Beta Regio was one of the few areas where fresh, dense rocks, possibily recently erupted basaltic volcanics, were exposed at the surface.

Not all those studying Venus are reconciled to the absence of plate tectonic processes, however, and the debate continued at the Houston meeting. On the one hand, H. Spetzler and K.A. Goettel argued for a weak crust^{3,4} and J.L. Warner provided a mechanism⁵ for recycling the crust via the subsidence of interlavered volcanic rocks and aeolian sediments; in contrast, S. Solomon reviewed the reasons6 why we should not yet rule out any of the possible styles of tectonism proposed for Venus. In an attempt to sidestep the issue of tectonic regimes, L. Wilson and J.B. Garvin^{7,8} showed that, irrespective of magma chemistry, eruptions on Venus were likely to produce lava flows longer than those on Earth. Because of the high surface pressure on Venus (55-90 bars), explosive eruptions which produce dispersed ash layers could only occur if the volatile contents of erupting magmas were high by terrestrial standards, exceeding about three weight per cent.

The new Soviet results bear on all aspects of the topics outlined above. Highresolution images (showing millimetresized particles in the foreground) were obtained through red, green and blue filters at both landing sites and colour composites produced. In each case, the sky appeared to have a yellow hue while exposed rock surfaces were a yellowish orange and fine-grained materials showed a greenish tint. A drilling device obtained samples (thought to be mainly low-strength soils) from the vicinity of each lander and transferred them into an X-ray fluorimeter

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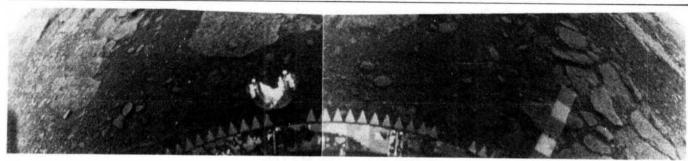
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^{*}The 13th Lunar and Planetary Science Conference was held at the Lyndon B Johnson Space Center Houston, Texas, on 15-19 March 1982



A picture of the surface of Venus from the USSR Venera 13.

system for major element chemical analysis (Table 1).

Table 1 Oxide analyses of Venus surface rock in weight per cent

Oxide	Venera	Venera
	13 site	14 site
SiO ₂	45	49
$Al_2\tilde{O}_3$	16	18
MgO	10	8
FeO	9	9
CaO	7	10
K ₂ O	4	0.2
TiO ₂	1.5	1.2
MnÕ	0.2	0.16

At the Venera 13 site, on rolling uplands high on the flanks of Beta Regio, extensive coherent strata are seen, together with isolated rocks showing some evidence of erosion. The composition of the rock sample is close to that typical of terrestrial

alkali basalts found in continental rift zones - a striking result when one recalls the proposed origin of Beta Regio as a rifted uplift over a hot-spot. Alkali basalts on Earth are associated with magma source regions rich in CO2 and the existence of such rocks on Venus lends support to the idea that CO₂-driven explosive volcanism may occur there despite the high surface pressure.

The surface at the Venera 14 landing site, in a lowland area south-east of Beta Regio. is dominated by extensive horizontal strata, 10-100 mm thick, which have the appearance of lithified sediments in the process of being eroded. The composition, however, is again very close to that of a terrestrial primary volcanic product, in this case an ocean-floor tholeiitic basalt. It is tempting to reconcile the composition with the sedimentary character of the rock by assuming that it is some kind of pyroclastic material, but then the problem of finding

sufficient volatiles to drive the eruption reappears. We should not jump to the conclusion that the tholeiitic nature of the Venera 14 rock necessarily implies that it was produced in a plate tectonic environment, as such a rock type would be on Earth. Indeed, too much must not be inferred about the interior of Venus from just two rock analyses; but it is ironic that each of the two analyses available, interpreted naively, supports one of the two main theories of Venus crustal structure.

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Punctuationism and Darwinism reconciled?

The Lake Turkana mollusc sequence

On 8 October, 1981 P G Williamson1 presented an extraordinarily "complete page in the history of evolution" in a very detailed sequence of fossil molluscs from the the eastern Turkana basin in East Africa. All of 13 lineages, including ten which could be followed for 4-5 million years, showed a pattern of evolution conforming to the 'punctuated equilbrium' model: change was concentrated in short (5,000-50,000 years) speciation events, between long periods (3-5 million years) of morphological stasis. How is such a pattern to be

In the same issue, Jones² pointed out that the rapidity with which change occurred is certainly no greater than can be brought about by conventional Darwinian selection. The real problem, as Williamson3 and others4 have made clear, is to explain the very long periods of stasis. Williamson favours the view, originally put forward by Eldredge and Gould⁵, that stasis is primarily the result of "developmental constraint", which makes certain kinds of developmental change difficult or impossible, and that speciation must necessarily involve the dismantling of these mechanisms. He sees evidence of this process in the periods of extreme developmental instability (recorded as an increase in phenotypic variance) that accompany speciation events in his mollusc sequences.

A contrasting view is that stasis may be explained by stabilizing selection — that is selection favouring the typical members of a population rather than the extremes. If this explanation is correct, then as Maynard Smith points out in a new book (Evolution Now*) what we need is "a theory which says something about selection, and hence about the environment. Since the major component of the environment of most species consists of other species in the ecosystem, it follows that we need a theory of ecosystems in which the component species are evolving by natural selection."

Williamson's articles have stimulated a great deal of comment, a small part of which appears below. Questions are raised both as to the nature of the pattern recorded in the fossil lineages — whether or not the bursts of rapid change are truly genetic in origin — and its implications. The notion of developmental constraint is debated and more general points, such as whether the 'punctuated equilibrium' pattern could be brought about by varying rates of environmental change, or whether it could be an artefact of sampling time scales, are also examined.

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Questions concerning speciation

from Ernst Mayr

THE pattern of evolution in the mollusc lineages reported by Williamson seems to be in excellent agreement with the theory of punctuated equilibria, which is based on the well-known paleontological observation that new species usually enter the fossil record abruptly (in geological time) and postulates that they persist subsequently with little significant change until extinction. The rapid change under the stress conditions of an evaporating Lake Turkana is, as Williamson points out, less of a puzzle than the millions of years of stasis.

Williamson refers to my observation that many currently living species show phenotypic uniformity even though their range extends over areas of considerable ecological and climatic diversity. This observation strengthens the thesis that it is not stabilizing selection of a set of essentially additive genes that is responsible for the stasis but rather the strength of the "internal balance" (Mather), "genetic homeostasis" (Lerner), "cohesion of the genotype" (Mayr). These authors postulate that the genotype as a whole is a finely balanced system, in which appropriate feedback mechanisms maintain morphological stability by compensating for whatever genetic changes occur through time at individual loci.

Williamson has presented a plausible explanation of his findings and it might well be the correct one. However, in his short communication, he has not refuted conceivable alternate theories nor has he provided answers to some disturbing questions that might arise in the mind of the reader.

First, Williamson states that the changes in 12 lines of sexually reproducing molluscs are paralleled exactly by events in the lineage of the asexually reproducing species Melanoides tuberculatus. Since an asexual phyletic lineage consists of hundreds, if not many thousands, of independent clones, how does Williamson explain that all of them experience a parallel history of equivalent genetic changes? This concordant phenotypic change of the scores of asexual clones suggests that the changes are not genetic at all, but merely modifications of the phenotype (see also the letter below from A.J. Boucot).

Second, does the variability of the asexual (*Melanoides*) lineage, consisting of numerous independent clones, differ from those of the sexual lineages?

Third, Williamson states that the new 'species' become suddenly extinct when the

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widespread parental species reinvade the Turkana basin. There are four sets of possibilities for the behaviour of parental and daughter "species" at the time of the establishment of sympatry: (a) Conditions in the Turkana basin, before the reinvasion by the widespread parental stocks, had deteriorated to such an extent that the new "species" had all become extinct already; (b) They had become reproductively isolated; in that case it should be possible to find them sympatrically in zones of contact: (c) They had not yet developed reproductive isolation and it should be possible to find hybrid populations with greatly increased variability; (d) The observed morphological differences of mother and daughter 'species' had no genetic basis but were merely phenotypic responses to different environmental conditions; in that case a sudden and total change of the phenotype should accompany the replacement of the lake waters. A finegrained analysis of the populations occurring during the crucial period of reversion to standard conditions should permit an answer to these questions.

Finally, one would also like to know more about one set of puzzling speciations. In six of the 13 lineages a second species

apparently originated in the lower Koobi Fora Formation (Bellomya unicolor, Cleopatra ferruginea, Melanoides tuberculatus, Caelatura bakeri, Mutela nilotica, and Eupera ferruginea). All six of these daughter species seem to become extinct before the end of the lower Koobi Fora (see Fig. 4). Do these six new daughter species coexist sympatrically with the parent stock without producing intermediates? Does Williamson believe that they originated in some peripheral isolate (a temporarily isolated lagoon?) and were able, after a rise in lake level, to invade the range of the parent stock? Several other questions are obvious, but one can not even begin to answer them until Williamson produces more factual evidence about these six postulated speciation events.

Williamson states that, on the whole, his findings are consistent with my theory of peripatric speciation in peripheral founder populations, with two drastic exceptions: The asexual Melanoides behaves like the sexual lineages, a fact not explained by Mayr's postulate of genetic revolutions, and the size of the shell deposits indicates that the 13 (19) lineages never truly seem to have passed through a narrow populational bottleneck, as demanded by the theory of peripatric speciation. It is impossible to resolve this conflict -Williamson himself does not try to do it until much better factual evidence for some of the events in the sequence of these mollusc faunas is presented.

Ecophenotypic or genotypic?

from Arthur J. Boucot

WILLIAMSON's brief comment (p. 441) that the presumably derivative morphologies, all arrayed in two, well-dated, very discrete horizons and one more diffuse horizon, cannot be considered to be ecophenotypic morphologies of the unchanging, longranging forms - many of which persist to the present — needs discussion. It is essential that in an area where lake waters of widely different composition are known to have occurred in the past, as well as the present, that the possibility of ecophenotypic, as contrasted with a genetic, change be dismissed only when adequate data has accumulated. One would have hoped that Williamson would have provided us with observational data, based on those species that persist to the present, showing that under conditions of varied lake water composition (both concentration and composition) there are no ecophenotypic effects.

If such data are unavailable, Williamson could have cultured at least some of the living species under conditions of salinity and water composition that might have

occurred in parts of the Turkana Basin region during the time interval studied. Without such data one must be forgiven for still considering that the possibility of ecophenotypic change is fully as rational as that of speciation. Few neontologists would adhere to the tenet that distinctly different skeletal morphologies within a genus invariably indicate different genotypes. Paleontologists normally assign distinctly different morphologies within a genus to different species, lacking evidence for a well documented morphologic gradient of ecophenotypic type, only because they commonly deal with extinct genera; not because they feel that the procedure is invariably defensible.

Finally, Williamson really should have provided us with a satisfying explanation of why speciational events should have occurred simultaneously, at two distinct

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and one diffuse horizon, within a variety of unrelated molluscs. This certainly is a strange coincidence from an evolutionary point of view, although entirely plausible from the ecophenotypic point of view if a major change in lake water chemistry and concentration affecting an arm of a large

lake was involved. The long stratigraphic range of those stocks still represented today makes it clear that they were present in the region throughout the entire time interval, whereas all of the morphologies derived from them during the brief intervals of morphologic change

lasted a very short time — this situation is entirely consistent with either local speciation under unknown physical conditions, or under locally isolated conditions involving different enough conditions to produce distinctive ecophenotypes.

Morphological stasis and developmental constraint: no problem for Neo-Darwinism

from Brian Charlesworth and Russell Lande

THE critical issue stemming from Williamson's papers is the explanation of stasis and increased variability associated with the episodes of change. The view he holds, that stasis is due to developmental constraints2, is equivalent to saying that the characters concerned lack genetic variability, so that selection is ineffective. But there is evidence for substantial levels. of heritable variability in most morphological characters that have been studied1,4, including snail shell traits5,6.

Stasis cannot, therefore, be explained by developmental constraints; either the characters are selectively neutral and population sizes are too large for genetic drift to be effective, or natural selection acts towards an intermediate optimum phenotype. Direct evidence for such stabilising selection on shell characters in snails was provided by the early biometricians^{7,8}, and similar data is available from many other traits9. Williamson is also incorrect in implying that stabilising selection has only recently been invoked by neo-Darwinists as an explanation of stasis; for example, Simpson (ref. 10, pp148-150 and 327-355) discusses this question at length.

The observation of increased variability

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at times of rapid change could have several explanations not discussed by Williamson. He neglects the possibility that it could be caused by a direct developmental response to increased heterogeneity of the habitat, or by the effects of temporal fluctuations during individual lifetimes, at periods when the environment is changing. Furthermore, since fossils classed as belonging to one geological horizon almost certainly span many generations, rapid evolutionary change over the period of sampling could generate increased variation. For these reasons, we are not convinced that the increased variation is a genuine evolutionary phenomenon. But even if this is granted, there would be no difficulty for neo-Darwinism. Selection experiments have demonstrated that stabilising selection can enhance developmental stability, or zones of

'canalisation', around an optimum phenotype by altering the patterns of genetic and non-genetic variation¹¹⁻¹³. Relaxation of stabilising selection14, and/or directional selection away from a zone of canalisation15-17, would then be predicted to produce an increase in phenotypic variability, until the population is recanalised by stabilising selection around a new optimum phenotype¹⁸ (Kirkpatrick, Am. Nat., in press). However, many characters under weak stabilising natural selection are not canalised in their appreciably development, and can be artificially selected to change a great deal without substantially increasing their variability4. The exact temporal patterns of variability expected thus depend on factors whose relative weights are difficult to assess for fossil material.

Are 'punctuations' artefacts of time-scales?

from Lev R. Ginzburg and Jay D. Rost

WE would like to give a brief explanation of why the 'punctuated' pattern of evolutionary change seen in Williamson's and other, less well documented, findings may well be an artefact of the sampling time scales. A more detailed argument is presented elsewhere¹.

Consider the following imaginary example: A population of E. coli is cultivated in a chemostat under a fixed environment for a number of years. Assume that we sample cells from the culture with different time intervals to discover whether the culture has evolved or not with respect to a quantitative trait. If we do this, biweekly, for instance; we will find most of the time that nothing has happened. Occasionally will we find changes that appear as punctuational, since the replacement time in a chemostat is typically much shorter than two weeks. Now, if we make our observations hourly, the details of the replacement process will be obvious and the evolutionary change will appear gradual. If we make only yearly observations, the process may look gradual for a different reason; we would average a number of changes during the year into one vearly change.

If we repeat the experiment with a number of isolated chemostats, and carry out bi-weekly sampling for a period of a few months, we expect most of the populations to show no change, but some of them will evolve away from the majority. This may look like a punctuative "speciation event." Too fine or too coarse a time scale will always lead to a more "gradual" picture, whereas at some intermediate scale, the process will appear as "punctuational." In the work of Williamson the mean time scale resolution is between 30,000 and 35,000 years. (The value is the sum of the time spans of segments 2, 3, 4 and 5, about 2.5 Myr,

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divided by 79 sample points). If a typical replacement time is significantly less (selective advantages of $10^{-4}-10^{-5}$ per year), our hypothesis may well be correct. Note that other well-documented studies claiming support for gradualism appear to have scale resolutions significantly greater than this. These researchers may be sampling at too coarse a time scale to detect 'punctuational' events (See Table below).

			Table	
	support of	(tot	ne-scale resolution al time span/total of samples)(yr)	Organism
b	Cronin et a Ozawa ³	L.2	,	Hominids Foraminiferans
	Gingerich ⁴ Kellogg ⁵		90,300 74,700	Condylarths Radiolarians

a, 625,000 = 2.5 Myr time span divided by four sample points (measuring cranial capacity). Includes specimens of Australopithecus africanus, Homo habilis, Homo erectus and Homo sapiens. b, 440,000 = 15Myr time span divided by 34 sample points. c, 90,300 = 3.07 Myr time span divided by 34 sample points; Hyopsodus. d, 74,700 = 2.54 Myr time span divided by 34 sample points. All total time spans were estimated from author's graphs.

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Punctuated equilibria and punctuated environments

from D.W. Lindsay

EVOLUTION is not an event which occurs without external agency. A selective pressure is necessary for evolution to occur and must be an important determinant of the rate of evolution. Where such rates appear to vary, as Williamson's study shows, surely the first aspect of the problem to receive attention should be the selective pressures involved. In a stable lacustrine environment, as seems to have existed between the bouts of 'speciation' described by Williamson, selective pressures may have been low or minimal so that a lack of change in a few correlated characters seems rather unsurprising. Equally unsurprising are sudden series of changes coinciding with 'major lacustrine regressions'. These would clearly introduce marked selective pressures and a consequent flurry of genotypic and phenotypic experimentation until adaptation had been attained, or not,

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and the environment stabilised. Thus, observations of stasis interspersed with rapid change may simply reflect changes in the direction and intensity of the selective pressures which are implied by the stratigraphic record. Generally, we ought to consider a punctuated environment in thoughts on punctuated environment in thoughts on punctuated evolution. It is a sad fact of paleontology that the conditions of stable, prolonged sedimentation which favour preservation may lead to low rates of evolution, while periods of environmental hiatus which would have accelerated the process may often be lost in the unconformities.

Williamson replies:

In answer to Mayr's first point: It is surely unneccessary to postulate that all the clones of Melanoides present in the Turkana Basin underwent parallel genetic changes during the episodes of morphological transformation at the Suregei and Guomde levels. It seems more probable that one, or a few, such clones made an appropriate evolutionary response to the relevant selective pressures, and that, as these few clones evolved, they proceeded to outcompete other 'conspecific' clones. Such interclonal competition is well documented, and Mayr himself has suggested competitive elimination of inferior clones as the probable mechanism whereby discontinuities between closely related asexual 'species' arise2. I address Mayr's further suggestion (that the transformations morphological documented in the Turkana sequence are purely ecophenotypic reactions) below.

In response to Mayr's second point: The variability (c.v.) of all measured traits in populations of the asexual taxon *Melanoides tuberculata* are quite comparable to the variabilities documented in typical populations of the gonochoristic gastropod taxa³.

Mayr's third point: The details surrounding the sudden reinvasion of parental stocks into the basin above the Suregei Tuff level speciation event, synchronous with a major lacustrine transgression, are at present poorly understood. Some 2-3 metres of fossilbarren sediment separates the last of the novel Suregei level forms from the first of the returning ancestral stocks. No hybrids, sympatric faunas or intermediates are currently known. It is not therefore possible to distinguish between Mayr's options (a), (b) and (c). But as discussed below, Mayr's option (d) appears untenable.

Mayr is correct in noting a set of speciation events in certain lineages in the Lower Member of the Koobi Fora Forma-

tion. These new forms appear suddenly in the record and coexist with their ancestral stocks; the event is therefore a modest adaptive radiation typical of that seen in many modern rift lakes. All these new endemics become extinct by the top of the Lower Member, due to a climatically induced regression — and consequent increase in alkalinity — well documented in the uppermost part of the Lower Member. It is indeed likely, that these new endemics arose in peripheral isolates of the large Lower Member lake, perhaps in the manner suggested by Hubendick⁴.

With reference to Mayr's last paragraph: I believe that it is indeed possible to resolve the conflict between Mayr's model of peripatric speciation and my observations in the Turkana Basin. I hope to address this topic in a forthcoming paper.

Boucot — and also Mayr — suggest that the novel forms I document as arising at the Suregei, Guomde, and Lower Member levels may simply be ecophenotypic 'reactive forms' of the 'ancestral' lineages. Several factors strongly indicate that this is not the case: (1) the novel forms arising in the Lower Member coexist with their presumed ancestral forms, and occur with them in the same 'life-assemblages'. The Lower Member novelties at least can not be simple ecophenotypic variants⁵. (2) The principle stem lineages in the Turkana Basin sequence are still extant and widely distributed in Africa, but even the most extreme modern environments in which these lineages occur at present produce a simple dwarfing of characteristic morphology (or in extremis, extinction of local populations), they never produce the striking reorganization of phenotype documented in the Turkana Basin sequence⁵. (3) The morphological transformations documented at the Suregei Tuff level are invariably unidirectional in character space, and required some 103-104 generations to accomplish6. This is hardly the nature or time-scale of a conventional ecophenotypic response⁵ The magnitude of phenotypic change during the Suregei and Guomde Level episodes is striking. In the bivalves, these changes involve a radical restructuring of shell form and hinge dentition, and major changes in the disposition of the muscle scars. The magnitude of the changes documented in both bivalves and gastropods is generally far greater than that observed in the ecophenotypic transformations of even the most plastic of modern African freshwater molluscs³. I do not therefore believe that the profound morphological transformations documented in the Turkana Basin sequence can be simply 'written off' as an ecophenotypic response.

Boucot expresses surprise that all lineages at the Suregei and Guomde levels speciate. But it has long been acknowledged that two major 'triggers' for speciation events within peripheral isolates are (1) isolation per se and (2)

environmental stress7. The Suregei and Guomde Level speciation events occur at times of lacustrine regression. The various molluscan lineages have similar vagilities, and occupy similar environments. All would therefore experience both isolation and stress during a major regressive phase. A broadly synchronous evolutionary response in all lineages is therefore reasonable.

Charlesworth and Lande are incorrect in assuming that evidence for substantial heritable variability in morphological characters implies that morphological stasis cannot be attributed primarily to developmental constraint. The question is not so much whether variation exists for selection to work on, but rather what, if anything, selection can actually make of this variation in nature. Developmental constraint may well block long-term directional morphological trends in large populations, even though the genetic potential for such evolutionary transformation exists. This is at least one tenable explanation for the fact that, in the record, such long-term morphological trends are rare, or absent, in continuous phyletic sequences.

Morphological stasis is not, therefore, a reflection of selective neutrality of phenotypes or simple stabilizing selection. The idea that most phenotypes are selectively neutral is not widely held, nor supported by Charlesworth and Lande's own references8. Neither is simple stabilizing selection an adequate explanation for long term morphological stasis. Many species - perhaps most exhibit stasis for millions of years. The idea that such species experience an unchanged selection regime for these immense spans of geological time seems inherently unlikely. Conventional neo-Darwinists have similarly recognised that simple stabilizing selection is an inadequate explanation for the analagous situation of range-wide phenotypic coherence in modern species; they therefore invoke developmental constraints of one form or another to explain this coherence9. In asserting such constraints to be the principal element in long-term temporal stasis, punctuationists are following the lead of conventional neo-Darwinian students of geographic variation.

Certain neo-Darwinists have recognised the primacy of developmental constraint in maintaining evolutionary stasis. For example Rendel¹⁰ states that "when a canalised character is to be changed the unfitness introduced when the fine adjustment between developmental processes is destroyed will always be counteracting directional selection . . . directional selection must always introduce unfitness. This antagonism between fitness and directional selection will have to be taken into account in interpreting long [term] stability. . .'

Charlesworth and Lande suggest that the greatly enhanced phenotypic variability I

document during periods of morphological transformation in the Turkana sequence simply represents a direct developmental response to increased heterogeneity of the environment. No evidence of any similar response is known from any modern population of the lineages concerned, even in the extremely wide range of environments many of them currently occupy; it hardly seems necessary to invoke them in this case. They then suggest that this variability might be due to 'mixing' of more- or -less evolved and stratigraphically adjacent populations. But as I point out5, the faunal units concerned seem to be undisturbed life assemblages with no evidence of reworking, and much of the variability derives from the presence of phenodeviants bearing as little relationship to the derived species as they do to the ancestral form. This does not suggest that the increased variability I document is due to the mixing of the products of a conventional neo-Darwinist evolutionary 'march of means'. It suggests pronounced developmental instability in the transitional forms. Charlesworth and Lande finally concede that this increased variability may represent developmental instability resulting from strong directional selection away from a zone of canalization - which is, after all, the explanation I offered in my original paper.

Ginzburg and Rost are correct in considering the gradualist and punctuational models to be, in part at least, alternate hypothesis about the distribution of rates of evolutionary change. But the critical issue in this context is the extent to which observed rates of change in a given interval can be extrapolated up into rates of change occurring in any longer time interval.

If Ginzburg and Rost's bacteria were evolving according to the gradualist model, the amount of change observed over any given sample interval could be extrapolated up to account for the net change occurring over any larger sample interval. If the bacteria were evolving according to the punctuational model, the amount of change observed over any given sample interval could not be extrapolated up into the net amount of change observed over a much larger interval. In the latter case, if a given sample interval happened to include a 'speciation event', estimated rates of net change over a larger sample interval would be much too high (long periods of stasis would occupy most of a longer time period). If, more likely, one happened to sample a period of stasis, extrapolated rates of change for a larger sample interval would be much too low, that is effectively zero.

The sampling time scale is therefore irrelevant to the punctuationist-gradualist controversy. In any case, much more than simple differences in rate of evolutionary

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100 YEARS AGO

On Tuesday evening, April 11, the public thoroughfare stretching between Hatton Garden and the Old Bailey was lighted for the first time by the electric light. The novelty of the installation was the fact that the incandescent system had been adopted in preference to the arc system. The Holborn street lamps each contain two of Edison's bulbs suspended from a cross bar running through the top of the lantern. The light is of a golden tinge like gas, but much purer, brighter, and steadier. The lamps were switched on and off with the greatest ease, and altogether the experiment was a complete success.

Mount Etna has again been in an active condition. An eruption and a rain of ashes (rampilli) has quite recently alarmed the neighbouring inhabitants.

from Nature 25, 564-5; April 13, 1882.

change are involved in the differences between the punctuated and gradualist models: for example, the size of the populations in which significant evolutionary changes is thought to occur is also a critical distinction between these two models11.

Lindsay suggests that selection pressure is a sine qua non of evolutionary change. He ignores such staples of conventional neo-Darwinism as founder effect, genetic drift, mutation pressure and the appearance of 'super fecund' mutants, all of which phenomena are thought to effect evolutionary change irrespective of — or even in opposition to — selection pressure. But Lindsay ignores these, and suggests that the 'variation in rates' I observe in the Turkana section are the simple consequence of variations in the Suregei and Guomde Level although speciation events occupy only $5 \times 10^3 - 5 \times 10^4$ yrs represented by the Turkana sequence. In other words, according to Lindsay, for only 1 per cent or 0.1 per cent of the time represented by the Turkana Basin sequence were selection pressures adequate to elicit any evolutionary response in any lineage. For the remaining 99 per cent or 99.9 per cent of the time, selection pressures were 'so low or minimal' - for 5 Myr - that no significant change occurred whatsoever in any lineage. Lindsay describes this as unsurprising. I find it incredible! In fact, even the most conventional of neo-Darwinists would agree that rates of evolutionary change are controlled by many more factors than simple fluctuation in selection pressures.

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REVIEW ARTICLE

The role of protein phosphorylation in neural and hormonal control of cellular activity

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Protein phosphorylation is now recognized to be the major general mechanism by which intracellular events in mammalian tissues are controlled by external physiological stimuli. However, only recently has the idea that different cellular functions are controlled by common protein kinases and protein phosphatases started to gain widespread acceptance. Thus there is an integrated network of regulatory pathways, mediated by phosphorylation—dephosphorylation, that allows diverse cellular events to be coordinated by neural and hormonal stimuli. The evidence that supports this concept is reviewed, with emphasis on the role of protein phosphorylation in enzyme regulation.

THE presence of phosphorus in proteins has been known for almost 100 years, but its importance has only been realized since the discovery of enzyme regulation by reversible phosphorylation. The current excitement stems from the work of Krebs, Fischer and Larner over the period 1955–70, when they discovered that the neural and hormonal control of glycogen metabolism in skeletal muscle was mediated by changes in the phosphorylation state of glycogen phosphorylase¹, phosphorylase kinase² and glycogen synthase³ (Fig. 1).

These three enzymes remained the only examples of this phenomenon until the late 1960s, but the situation changed rapidly following the discovery of cyclic AMP-dependent protein kinase (cAMP-PrK)⁴. The past 10 years have seen an extraordinary and still accelerating growth in this area. About 35 enzymes and countless other proteins are now known to be regulated in this manner, and protein phosphorylation is clearly the major general mechanism by which intracellular events respond to external physiological stimuli⁵.

The first part of this review will summarize current knowledge of the control of glycogen metabolism, as this system is understood in the greatest molecular detail and continues to act as a model with which others are compared. The second part will present the increasing body of evidence that implicates many of the proteins involved in the control of glycogen metabolism in the regulation of other cellular activities.

Neural and hormonal control of glycogen metabolism in mammalian skeletal muscle

Glycogen is a major source of energy for muscle contraction. Its breakdown and synthesis are regulated by the contractile state of the tissue as well as by the hormones adrenaline and insulin, through changes in the phosphorylation state of glycogen phosphorylase and glycogen synthase (Fig. 1). These interconversion reactions are now known to involve five different protein kinases, four protein phosphatases, and regulatory proteins such as calmodulin, troponin-C, inhibitor-1 and inhibitor-2. Some of these proteins allow glycogen metabolism to respond to the second messengers Ca²⁺ and cyclic AMP (Fig. 1). The role of other proteins is less clear, although they may be involved in the action of insulin. Recent advances in understanding the molecular mechanisms underlying these reactions are summarized below.

Neural control via calcium ions: When muscle is stimulated electrically, calcium ions released from the sarcoplasmic

reticulum not only initiate muscle contraction but also activate phosphorylase kinase^{6,7}. As a result, the phosphorylation state of glycogen phosphorylase is increased⁸⁻¹⁰ and glycogenolysis is accelerated to provide the ATP required to sustain muscle contraction. Recently, phosphorylase kinase has been shown to phosphorylate glycogen synthase and to decrease its activity¹¹⁻¹⁵. Thus, the two opposing pathways of glycogenolysis and glycogen synthesis may be regulated in a synchronous manner during contraction.

Phosphorylase kinase is the key to both the neural and hormonal mechanisms for stimulating glycogenolysis, as it is not only dependent on Ca^{2+} , but is also a target for cAMP-PrK (Fig. 1). The enzyme has the subunit structure $(\alpha\beta\gamma\delta)_4$, where the α - and β -subunits are the components phosphorylated by cAMP-PrK 16,17 , the γ -subunit seems to be the catalytic subunit 18 and the δ -subunit is the Ca^{2+} -binding subunit 19,20 . The δ -subunit is identical to calmodulin $^{19-22}$, a protein

The δ -subunit is identical to calmodulin ¹⁹⁻²², a protein originally discovered as an activator of a quite different enzyme but which is now implicated in the regulation of many Ca²⁺-dependent enzymes (discussed below). Calmodulin binds four calcium ions per mol at micromolar concentrations²³, the affinity of the two Ca²⁺-binding sites in the N-terminal portion being 10-fold higher than those in the C-terminal domain²⁴.

Phosphorylation by cAMP-PrK activates phosphorylase kinase \sim 15-20-fold at saturating Ca²⁺ concentrations and decreases the K_a for Ca²⁺ \sim 15-fold²⁵. Thus, phosphorylation not only enhances the activity of the γ -subunit, but may also allow activation to occur when fewer calcium ions are bound to calmodulin. This suggests a biological role for the different classes of Ca²⁺-binding sites in calmodulin²⁶.

Phosphorylase kinase has been found to interact with a second molecule of calmodulin, termed the δ' -subunit, which only binds to the enzyme in the presence of Ca^{2+} where it interacts with the α - and β -subunits. In contrast, the integral molecule of calmodulin (the δ -subunit) remains complexed with the γ -subunit even in the absence of Ca^{2+} (refs 20, 27, 28). The δ' -subunit strongly activates the dephosphorylated form of phosphorylase kinase but has little or no effect on the phosphorylated form²⁵.

Calmodulin is closely related in structure and Ca^{2+} -binding properties to troponin- C^{22} , the protein on the thin filaments of muscle that confers Ca^{2+} sensitivity to the contractile apparatus. Troponin-C, the troponin complex, and even artificial thin filaments of muscle, can substitute for the δ' -subunit, and several lines of evidence suggest that troponin-C rather than

Table 1 Classification of protein phosphatases in cellular regulation

Туре	Protein phosphatase	Inhibited by I ₁ and I ₂	Specificity for phosphorylase kinase	Substrate specificity	Regulators
1	Protein phosphatase-1	Yes	β-subunit	Broad	$I_1, I_2, GSK-3+Mg^{2+}-ATP$
2	Protein phosphatase-2A	No	α-subunit	Broad	Unknown
2	Protein phosphatase-2B	No	α -subunit	Narrow	Ca ²⁺ calmodulin
2	Protein phosphatase-2C	No	α -subunit	Broad	Mg ²⁺

Protein phosphatases-2B and -2C are completely dependent on Ca^{2+} and Mg^{2+} , respectively, whereas protein phosphatases-1 and -2A are active in the absence of divalent cations. Protein phosphatases-1 and -2A have very similar substrate specificities. The specificity of protein phosphatase-2C is also broad, although it does not act on some phosphoproteins such as phosphorylase a (refs 52-55). Protein phosphatase-2B has a very restricted specificity, and of many phosphoproteins examined, only phosphorylase kinase (α -subunit), inhibitor-1 and myosin light chains have so far been found to serve as substrates^{69,70}. Abbreviations: I_1 , I_2 , inhibitor-1 and inhibitor-2; GSK-3, glycogen synthase kinase-3.

the δ' -subunit may be the important activator in $vivo^{25,26}$. At physiological Ca²⁺ concentrations (1–3 μ M), the dephosphorylated form of phosphorylase kinase is activated ~20-fold by the troponin complex^{25,26}.

Figure 2 summarizes current ideas about the regulation of glycogen metabolism by Ca^{2+} . Phosphorylase kinase is controlled by two related Ca^{2+} -binding proteins, but their relative importance depends on the phosphorylation state of the enzyme. Troponin-C seems to be the dominant regulator of the dephosphorylated form, while calmodulin (the δ -subunit) determines the Ca^{2+} sensitivity of the phosphorylated species. Activation of glycogenolysis and muscle contraction by the same Ca^{2+} -binding protein (troponin-C) represents a simple mechanism for synchronizing these two processes, and perhaps this is why phosphorylase kinase is so large (molecular weight 1,300,000 ref. 17)), having to span the distance between the protein–glycogen complex and thin filaments if these ideas are correct. Knowledge of its precise intracellular location in contracting muscle will be required to test this hypothesis.

Hormonal control of glycogen metabolism by multi-site phosphorylation: It was perhaps fortunate that the first example of enzyme regulation by reversible phosphorylation (activation of glycogen phosphorylase by phosphorylase kinase) should involve phosphorylation at a single site by a single protein kinase, since it greatly facilitated elucidation of the complex

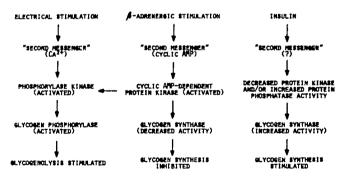


Fig. 1 Knowledge of the neural and hormonal control of glycogen metabolism in mammalian skeletal muscle up to 1970.

effects of covalent modification on the kinetic properties²⁹⁻³². This situation is relatively uncommon, however, and 'multi-site phosphorylation' is turning out to be the rule rather than the exception.

Phosphorylase kinase is phosphorylated on two serine residues by cAMP-PrK at physiological Mg^{2+} concentrations, one on the α -subunit and one on the β -subunit 16,17 . Both serine residues become phosphorylated in vivo in response to adrenaline Phosphorylation of the β -subunit occurs most rapidly in vitro, and activation correlates with the extent of phosphorylation of this subunit 17,33,34 . Phosphorylation of the α -subunit does not alter the activity 34,35 and its function is unclear. It was initially thought that phosphorylation of the α -subunit stimulated the rate at which the β -subunit was dephosphorylated 34,35 ,

but the experimental observations on which this hypothesis was based can now be explained by a quite different phenomenon³⁷.

Glycogen synthase is phosphorylated on seven serine residues by five different protein kinases, and all seven sites are phosphorylated *in vivo*. Cyclic AMP-dependent protein kinase phosphorylates sites 1a, 1b and 2 (ref. 38), phosphorylase kinase site 2 (ref. 12), glycogen synthase kinase-3 sites 3a, 3b and 3c (ref. 39), glycogen synthase kinase-4 site 2 and glycogen synthase kinase-5 site 5 (ref. 40). The organization of the sites in the polypeptide chain⁴¹ is shown in Fig. 3.

The effect of phosphorylation is to decrease the activity of glycogen synthase, although the changes in kinetic parameters are complex, and different phosphorylation sites have different effects. Phosphorylation increases the $K_{\rm m}$ for the substrate UDP-glucose, decreases the $K_{\rm l}$ for inhibitors such as inorganic phosphate and ADP, and increases the $K_{\rm l}$ for the activator glucose 6-phosphate⁴². Phosphorylation of sites (3a+3b+3c) influences the kinetic parameters to a greater extent than does phosphorylation of site 2 or site 1a, but the effects are additive so that even larger changes are produced when all five sites are phosphorylated^{40,43}. The phosphorylation of site 1b or site 5 does not seem to affect the kinetic properties^{38,40}.

In normally fed animals, glycogen synthase contains 3 mols of phosphate bound covalently to each subunit, with the phosphorylation of sites (3a+3b+3c) having a dominant role in determining the kinetic properties⁴⁴. Following the administration of adrenaline there are 5 mols of phosphate on each subunit of glycogen synthase⁴⁴⁻⁴⁶, and the activity of the enzyme is decreased mainly by increased phosphorylation at site 2 and sites $(3a+3b+3c)^{44}$.

The finding that site 2 becomes fully phosphorylated in response to adrenaline in conditions where site 1a phosphorylation is only increased slightly was unexpected, as site 1a is phosphorylated much more rapidly than site 2 by cAMP-PrK in vitro, and is dephosphorylated more slowly³⁸. The increased phosphorylation of site 2 may therefore be mediated indirectly by cAMP-PrK, through the activation of phosphorylase kinase (Fig. 3). Even more surprising was the finding that almost half of the increase in phosphate occurs at sites (3a+3b+3c), which are phosphorylated by glycogen synthase kinase-3. This enzyme is unaffected by cyclic AMP⁴³ and does not seem to be a substrate for cAMP-PrK (B. A. Hemmings and P. Cohen, unpublished work).

Recent studies indicate that the activation of glycogen synthase by insulin is largely explained by decreased phosphorylation of sites (3a+3b+3c) (P. J. Parker and P. Cohen, unpublished work). These results suggest that glycogen synthase kinase-3 may be controlled by insulin, which is particularly interesting in view of the surprising discovery that this protein kinase is also an activator of protein phosphatase-1, the major glycogen synthase phosphatase in skeletal muscle (see the following section).

Multi-site phosphorylation is a simple mechanism for greatly increasing the regulatory potential of enzymes and is being found to be a common occurrence. Phosphorylation at one site may amplify or even antagonize the effects of phosphorylation at other sites, or alter the rates at which they are phosphorylated

or dephosphorylated. The mitochondrial pyruvate dehydrogenase complex⁴⁷⁻⁴⁹ and retinal membrane protein rhodopsin^{50,51} are well established examples of this phenomenon. The phosphorylation of three serine residues on the α -subunit of pyruvate dehydrogenase may help to lock this enzyme into the inactive state in conditions such as insulin deprivation, thereby preventing the oxidation of glucose or its conversion to fatty acids. The phosphorylation of five serine and threonine residues at the C-terminus of rhodopsin may be involved in the regulation of dark-light adaptation. Phosphorylation at different sites by different protein kinases enables enzymes to respond to several physiological stimuli, and in such situations interactions between phosphorylation sites may frequently represent the mechanism by which one stimulus influences another. A number of examples which illustrate this idea will be described below. Protein phosphatases involved in glycogen metabolism in skeletal muscle: Four distinct enzymes, termed protein phosphatase-1 and protein phosphatases-2A, -2B and -2C (Table 1), have been identified in mammalian skeletal muscle and other tissues that are capable of dephosphorylating the enzymes of glycogen metabolism⁵²⁻⁵⁵. Protein phosphatase-1 specifically dephosphorylates the β -subunit of phosphorylase kinase and is powerfully inhibited by two proteins termed inhibitor-1 and inhibitor-2 (see below), whereas the three type-2 protein phos-

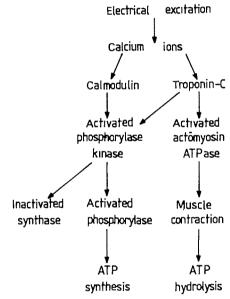


Fig. 2 Role of Ca²⁺, calmodulin and troponin in synchronizing glycogen breakdown, glycogen synthesis and muscle contraction.

phatases preferentially dephosphorylate the α -subunit of phosphorylase kinase and are unaffected by these inhibitor proteins. The concentrations of protein phosphatases-2A and -2C are low in skeletal muscle, and account for only a small proportion of the phosphatase activity towards the enzymes of glycogen metabolism in this tissue. These two enzymes are present at higher concentrations in the liver, and are likely to be more important in the regulation of other metabolic pathways (see below).

Protein phosphatase-1 is the most important enzyme in the regulation of glycogen metabolism in skeletal muscle³⁶, where it is the major phosphatase for glycogen synthase, glycogen phosphorylase and the β -subunit of phosphorylase kinase. A single enzyme therefore carries out each of the dephosphorylation reactions that inhibit glycogenolysis or activate glycogen synthesis (Fig. 4). It is becoming increasingly clear that protein phosphatase-1 is controlled in a variety of ways. A form of the enzyme has been isolated that is completely inactive until incubated with a further protein and Mg²⁺-ATP^{37,56-61}, and this activating protein has surprisingly turned out to be glycogen synthase kinase-3^{59,60}. Although there is not yet any evidence

that this activation system operates in vivo, an attractive idea is that it might be under the control of insulin⁵.

A further mechanism by which protein phosphatase-1 is regulated is through its interaction with the two thermostable proteins inhibitor-1 and inhibitor-2^{36,62-65}. It is of special interest that, only after phosphorylation on a threonine residue by cAMP-PrK^{56,60-62,65-68} can inhibitor-1 inhibit protein phosphatase-1 (Fig. 4). By such means inhibitor-1 provides a mechanism for amplifying the effects of cyclic AMP and for exerting control over several protein kinase-protein phosphatase cycles. Inhibitor-1 may also allow cyclic AMP to control enzymes that are phosphorylated by protein kinases other than cAMP-PrK. This would be the simplest explanation for the increased phosphorylation of glycogen synthase at sites (3a + 3b+3c) in response to adrenaline, and may also contribute to some of the increased phosphorylation at site 2.

Interestingly, inhibitor-1 does not inhibit its own dephosphorylation by protein phosphatase-1⁶⁴; consequently, protein phosphatase-1 could dephosphorylate inhibitor-1 in vivo. However, protein phosphatases 2A and 2B are also powerful inhibitor-1 phosphatases in vitro. It has recently been discovered that protein phosphatase-2B is a Ca²⁺-dependent enzyme that is stimulated 10-20-fold by calmodulin^{69,70}, suggesting that it may only be active during muscle contraction. The dephosphorylation of inhibitor-1 by protein phosphatase-2B, if it occurred in vivo, might therefore represent a mechanism for the activation of protein phosphatase-1 during contraction. This should stimulate the rate at which glycogen is resynthesized when contraction ceases, and allow the rate of glycogen synthesis in resting muscle to be linked to the strength and duration of the previous contraction.

Role of regulatory proteins of glycogen metabolism in other cellular processes

Cyclic AMP-dependent protein kinase: Although originally discovered as an activator of phosphorylase kinase⁴, it soon became obvious that cAMP-PrK must have a much wider role in mediating the diverse effects of cyclic AMP. The hypothesis arose⁷¹ that the specificity of a hormone is determined by whether its receptor is present on the plasma membrane of a

Table 2 Enzyme	s activated by calmod	ulin
Enzyme	Tissue '	Refs
High K _m cyclic nucleotide		
phosphodiesterase	Brain, heart	150-152
Adenylate cyclase	Brain	107
$(Ca^{2+} + Mg^{2+})ATPase$	Erythrocyte	153, 154
	and other	
	cell membranes	
Phosphorylase kinase	Muscle	19, 26, 28
Myosin kinase	Muscle and	94, 95
	non-muscle tissues	
Glycogen synthase kinase	Liver	108
Phospholamban kinase	Cardiac muscle	109
Tryptophan/tyrosine		
hydroxylase kinase	Brain	110, 155-157
Protein-I kinase	Brain	111, 112
Protein phosphatase-2B	Muscle, brain	70
NAD kinase	Sea urchin eggs,	158, 159
	higher plants	
Guanylate cyclase	Tetrahymena	160

target cell, and which physiological substrates for cAMP-PrK are present within those cells. In the case of substrates that are enzymes, the effect of phosphorylation is often to change the $K_{\rm m}$ for a substrate, the $K_{\rm a}$ for an activator or the $K_{\rm l}$ for an inhibitor, as clearly illustrated by the enzymes of glycogen metabolism. Substrates, activators and inhibitors may also affect the rate at which an enzyme is phosphorylated or dephosphorylated, thereby amplifying or suppressing the effects of covalent modification. Phosphorylation—dephosphorylation should not therefore be regarded as a mechanism for turning an enzyme

5

on or off but rather for switching it between two forms (or up to several hundred in the case of enzymes with complex subunit structures that exhibit multi-site phosphorylation) that respond differently to substrates and regulator molecules. The interplay between covalent phosphorylation, allosteric effector molecules and substrates represents the means by which extracellular (neural and hormonal) and intracellular information is integrated to determine the precise activity of a metabolic pathway in vivo.

One obvious prediction of this model is that many substrates for cAMP-PrK must exist in different cells to explain the great diversity of action of many hormones, and it has become increasingly clear that this is the case. A number of enzymes are regulated by cAMP-PrK in vitro and are likely to be physiological substrates for this enzyme. The activation of triglyceride lipase⁷² and inactivation of glycerol phosphate acyl transferase⁷³ may coordinate triglyceride breakdown and synthesis respectively in adipose tissue in response to adrenaline, in the same way that activation of phosphorylase kinase and inhibition of glycogen synthase allow coordinated control of glycogen breakdown and synthesis by adrenaline (skeletal muscle) or glucagon (liver). The activation of cholesterol esterase increases the pool of cholesterol for steroidogenesis in the adrenal cortex in response to ACTH stimulation⁷⁴. The phosphorylation of acetyl-CoA carboxylase decreases its activity and increases the K_a for the activator citrate⁷⁵. This underlies the inhibition of fatty acid synthesis in adipose tissue by adrenaline⁷⁶ and in the liver by glucagon⁷⁷. Acetyl-CoA carboxylase is phosphorylated at multiple sites and several protein kinases (including glycogen synthase kinase-5) seem to be involved^{40,75}. The inactivation of 6-phosphofructo 2-kinase^{78,79} and pyruvate kinase⁸⁰, and activation of fructose 1:6 bisphosphatase^{81,82}, appear to be crucial events in the stimulation of gluconeogenesis by glucagon in hepatic tissue. 6-Phosphofructo 2-kinase catalyses the formation of fructose 2:6 bisphosphate, a key activator of 6-phosphofructo 1-kinase and a powerful inhibitor of fructose 1:6 bisphosphatase^{83–86}

The phosphorylation of pyruvate kinase increases the K_m for phosphoenolpyruvate, makes it more sensitive to the inhibitors ATP and alanine, and increases the K_a for the activator fructose 1:6 bisphosphate⁸⁰. The phosphorylation of fructose 1:6 bisphosphatase decreases the K_m for fructose 1:6 bisphosphate⁸¹. The activation of hepatic phenylalanine hydroxylase⁸⁷⁻⁹⁰ is probably one of several mechanisms by which amino acid degradation is activated to provide gluconeogenic precursors and tricarboxylic acid cycle intermediates in response to glucagon. The activation of tyrosine hydroxylase in the adrenal gland or brain⁹¹⁻⁹³ increases the rate of synthesis of the hormones and neurotransmitters adrenaline, noradrenaline and dopamine in response to neural excitation. The Ca²⁺-calmodulin-dependent

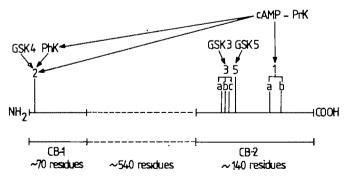


Fig. 3 Organization of the phosphorylation sites in rabbit skeletal muscle glycogen synthase. Site 2 is seven residues from the N-terminus and sites 3a, 3b, 3c, 5, 1a and 1b are 30, 34, 38, 46, 87 and 100 residues from the N-terminus of a large CNBr-peptide (CB-2) at the C-terminal end of glycogen synthase 41. Abbreviations: cAMP-PrK, cyclic AMP-dependent protein kinase; PhK, phosphorylase kinase; GSK, glycogen synthase kinase.

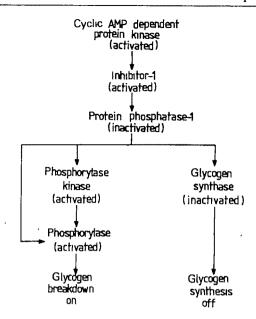


Fig. 4 Role of protein phosphatase-1 and inhibitor-1 in the β -adrenergic control of glycogen metabolism in mammalian skeletal muscle.

enzyme myosin light chain kinase is essential for the assembly of myosin into filaments and for actin-activated actomyosin ATPase activity in smooth muscle and non-muscle tissues⁹⁴⁻⁹⁶. The inactivation of this enzyme by cAMP-PrK⁹⁷ may be a key event in the adrenaline-induced relaxation of smooth muscle^{98,99}.

Of the 12 enzymes discussed above, only phosphorylase kinase, glycogen synthase and pyruvate kinase meet all four criteria necessary to establish that a protein serves as a physiological substrate for cAMP-PrK in vivo 100,101, although acetyl-CoA carboxylase 65,90 come very close to doing so.

Although it is likely that regulation by hormones of processes such as muscle contractility, secretion, membrane permeability and transport, growth and differentiation, protein induction and protein degradation also involve phosphorylation, progress is still hampered by a lack of understanding of the molecular nature of the processes themselves. Nevertheless, the importance of cAMP-PrK has been established in several cases. For example, the phosphorylation of troponin-I in cardiac muscle fibres decreases the affinity of troponin-C for Ca²⁺, and may contribute to the increased rate of relaxation of cardiac muscle produced by adrenaline⁹⁵. Similarly, the phosphorylation of a protein in cardiac sarcoplasmic reticulum, termed phospholamban, is associated with activation of the sarcoplasmic reticulum ATPase and increased rates of Ca²⁺ uptake into these vesicles^{95,102-104}. This may also promote the increased rate of relaxation of cardiac muscle by adrenaline. The growth of mammalian cells is inhibited by cyclic AMP after several days in culture, allowing the selection of mutant strains in which this phenomenon no longer occurs, and these mutant cells are invariably found to have defective cAMP-PrK activity 105,106. Thus, even the long-term effects of cyclic AMP on cell growth are clearly mediated by this protein kinase, and must involve the phosphorylation of as yet unidentified proteins.

It is important to stress that cAMP-PrK is a highly specific enzyme, phosphorylating very few proteins at significant rates. Even physiological substrates are only phosphorylated at one or two sites out of a large number of serine or threonine residues that are potentially available ¹⁰¹. The basis for this specificity has become clearer following the discovery that physiological substrates invariably contain two adjacent basic amino acids just N-terminal to the residue that is phosphorylated. This structural feature is now known to be critical for specific substrate recognition (reviewed in refs 5, 36, 80, 101).

Calmodulin: Neural and hormonal stimulation of a variety of cells produces transient rises in the cytoplasmic concentration , but only recently has it become clear that many of the biological actions of Ca²⁺ are mediated by the Ca²⁺-binding protein calmodulin, in a manner that closely resembles the action of cyclic AMP^{23,107}. The ability of calmodulin to function as a Ca2+-dependent regulator of enzyme activity is a consequence of conformational changes following its binding of Ca²⁺, which lead to the formation of specific interaction domains. Table 2 lists 12 enzymes that are activated by, or completely dependent on, calmodulin, These include a number of protein kinases or protein phosphatases, three of which (phosphorylase kinase, myosin light chain kinase and protein phosphatase-2B) have been discussed already. A calmodulindependent protein kinase in rat liver phosphorylates site 2 of glycogen synthase, but is distinct from phosphorylase kinase as it cannot activate glycogen phosphorylase¹⁰⁸. A cardiac protein kinase which phosphorylates phospholamban does so at a site(s) distinct from that phosphorylated by cAMP-PrK, and is associated with increased rates of Ca²⁺ uptake¹⁰⁹. A brain protein kinase phosphorylates tryptophan hydroxylase and tyrosine hydroxylase 110, and therefore regulates the synthesis of the neurotransmitters serotonin and dopamine. Protein-I, a major protein of the postsynaptic membrane, that may function in the control of neurotransmitter release, is a target for two different calcium-dependent protein kinases that phosphorylate distinct serine residues on the protein. Protein-I is also a target for cAMP-PrK^{111,112}. In addition, a variety of tissues contain membrane-bound calmodulin-dependent protein kinases that phosphorylate membrane proteins whose identity and function are as yet unknown 113,114

Although the cyclic AMP and calmodulin systems are formally analogous in the way in which they produce phosphorylation of a number of proteins (Fig. 5), a major difference between the two systems is that mammalian cells contain one catalytic subunit of cAMP-PrK that phosphorylates a variety of proteins¹⁰¹, whereas many different protein kinases with restricted substrate specificities are activated by calmodulin.

Another point, indicated by the broken lines in Fig. 5, is that the cyclic AMP and Ca^{2+} -calmodulin pathways are closely interlinked and may often be interchangeable in different tissues. Thus, adrenergic stimulation of glycogenolysis in skeletal muscle involves the β -receptors and is mediated by cyclic

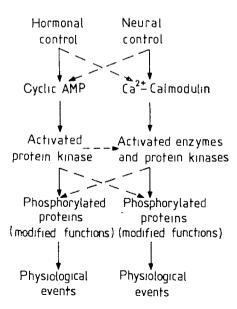


Fig. 5 Interrelationships between the cyclic AMP and Ca²⁺-calmodulin systems in the neural and hormonal control of cellular activity.

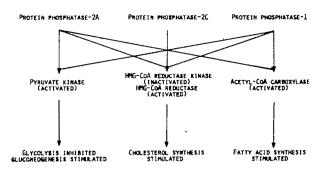


Fig. 6 Role of protein phosphatases-1, -2A and -2C in the regulation of gluconeogenesis, cholesterol synthesis and fatty acid synthesis.

AMP, whereas in adult rat liver it is an α -adrenergic effect mediated at least in part by Ca²⁺ (refs 115, 116). Similarly, electrical excitation of skeletal muscle elevates the cytoplasmic Ca²⁺ concentration, whereas it increases cyclic AMP as well as Ca²⁺ in the brain. The latter effect may result from activation of the calmodulin-dependent adenylate cyclase (Table 2) which is present in brain, but not in most other tissues.

A second point of interconnection between the cyclic AMP and Ca²⁺-calmodulin pathways is at the level of the protein kinases. Figure 5 suggests that cAMP-PrK may often phosphorylate calmodulin-dependent enzymes. The two well established examples of this phenomenon are phosphorylase kinase and myosin light-chain kinase. Further interconnection also occurs at the level of phosphorylated substrates. Figure 5 suggests that cyclic AMP and Ca²⁺-calmodulin-dependent protein kinases frequently phosphorylate the same proteins, although this will usually occur at distinct sites due to the high specificity of protein kinases (multi-site phosphorylation). Examples already discussed here are glycogen synthase, phospholamban, tyrosine hydroxylase and protein-I. Another example is 'goblin', a protein of molecular weight 230,000 in the plasma membrane of avian erythrocytes, that is implicated in the hormonal regulation of Na⁺-K⁺ co-transport¹¹⁷.

Although the importance of calmodulin in cellular regulation is firmly established, many fundamental questions remain unresolved. For example, the ability of calmodulin to activate an enzyme in vitro does not prove that this is a physiologically relevant reaction, and calmodulin may simply be substituting for another calcium-binding protein. Another question is whether different calmodulin-dependent enzymes bind to calmodulin in an identical manner, and if they do not118, might one molecule of calmodulin be able to activate several different enzymes simultaneously? Are different calmodulin-dependent enzymes activated by the same concentration of Ca2+ in vivo? This will depend not only on how many Ca2+-binding sites must be occupied in order to achieve activation, but on the amount of free calmodulin available to interact with any given enzyme^{25,119}, and on the changes in the Ca²⁺-binding properties of calmodulin that accompany its interaction with target proteins. Several calmodulin-dependent enzymes are apparently only activated when all four Ca²⁺-binding sites on calmodulin are fully occupied^{23,119}, but this requirement could be modified by phosphorylation or some other covalent modification as in the case of phosphorylase kinase. Note also that one enzyme can be regulated by two different Ca²⁺-binding proteins; this is the case for phosphorylase kinase and protein phosphatase-2B⁷⁰ and may apply frequently. All these possibilities demonstrate that the potential for very sophisticated control by Ca² exists; this could, in a sense, be regarded as the equivalent of multi-site phosphorylation.

The protein phosphatases involved in cellular regulation: One of the first pieces of evidence that implicated cAMP-PrK in metabolic processes other than glycogen metabolism, was its

high concentration in tissues where glycogen metabolism was of very minor importance. This is also the case for protein phosphatases-1, -2A, -2B and -2C in mammalian tissues 120,121. Indeed, current evidence suggests that protein phosphatases-2A, -2B and -2C may be more important in the control of metabolic pathways other than glycogen metabolism.

Protein phosphatases-1, -2A and -2C (Table 1) have broad substrate specificities⁵³⁻⁵⁵, and account for all the measurable protein phosphatase activity in mammalian liver towards L-pyruvate kinase, acetyl-CoA carboxylase, hydroxymethylglutaryl-CoA (HMG-CoA) reductase and HMG-CoA reductase kinase¹²²⁻¹²⁴, as well as the enzymes of glycogen metabolism. They therefore seem to be the major protein phosphatases involved in the control of gluconeogenesis, fatty acid synthesis and cholesterol synthesis (Fig. 6). The concentrations of protein phosphatases-2A and -2C are about threefold higher in liver than skeletal muscle^{55,121}, supporting the idea that they may be particularly important in the regulation of hepatic metabolism.

Protein phosphatases-2A and -2C have been isolated from smooth muscle as myosin light-chain phosphatases^{125,126}, and may be involved in the regulation of muscle contraction. However, protein phosphatase-1 and protein phosphatase-2B are also myosin light-chain phosphatases in vitro^{54,69}. Protein phosphatases-1 and -2A seem to be the major phosphatases acting on protein synthesis initiation factor eIF-2^{54,126} and may therefore function as stimulators of protein synthesis¹²⁷.

In view of the broad and overlapping substrate specificities of protein phosphatases-1, -2A and -2C in vitro, it is frequently difficult to assess the relative importance of these enzymes in the regulation of different metabolic pathways. In skeletal muscle, the concentrations of protein phosphatase-2A and protein phosphatase-2C are low, and it is difficult to believe that protein phosphatase-1 is not the major protein phosphatase involved in glycogen metabolism, particularly as it is specifically associated with glycogen. In the liver, the situation is much less clear. Protein phosphatase-1 is not only associated with glycogen. So, but also with microsomes 121, suggesting an involvement in processes such as cholesterol synthesis and protein synthesis. Protein phosphatases-2A and -2C are exclusively located in the liver cytosol 55,121. The former is very active towards the enzymes that regulate gluconeogenesis, fatty acid synthesis and cholesterol synthesis 122, while the latter is most active against the enzymes of cholesterol synthesis 54,122. However, these observations do not prove that they are the most important protein phosphatases acting on these substrates in vivo.

It was recently reported that microinjection of inhibitor-1 blocks the progesterone-induced division of *Xenopus* oocytes¹²⁸, suggesting that protein phosphatase-1 is responsible for a dephosphorylation event that is known to trigger the meiotic maturation of oocytes¹²⁹. Introduction of inhibitor-1 and inhibitor-2 into other cells could prove to be a powerful method for probing the biological functions of protein phosphatase-1.

The Ca²⁺-calmodulin-dependent protein phosphatase, protein phosphatase-2B (Table 1), is present at high concentrations in skeletal muscle and brain, but its concentration is low in liver^{55,121}. It appears to be identical to calcineurin (also termed CaM-BP₈₀), a major calmodulin-binding protein in brain^{70,130,131}, found in particularly high levels in the neostriatum¹³¹. Its immunohistochemical localization at postsynaptic densities and microtubules of postsynaptic dendrites¹³¹ suggests a role in the regulation of neurotransmitter action and microtubular function.

Protein phosphorylation and the control of cellular activity

The preceding sections have summarized some of the major lines of evidence that implicate cAMP-PrK, calmodulin and protein phosphatases-1, -2A, -2B and -2C in cellular processes

other than glycogen metabolism. Inhibitor-1, inhibitor-2 and glycogen synthase kinase-3 should also have more general roles as they control the activity of protein phosphatase-1. Thus, a wide range of cellular processes may respond to neural and hormonal stimuli by using remarkably similar regulatory networks that share common protein kinases, protein phosphatases and regulatory proteins.

Of course, many questions are far from being resolved. One concerns the role of 'silent' phosphorylation sites that do not appear to influence enzyme activities directly, such as the α -subunit of phosphorylase kinase or sites 1b and 5 of glycogen synthase. Two further examples are 6-phosphofructo 1-kinase^{132,133} and ATP-citrate lyase^{134,135} in rat liver. These enzymes are substrates for cAMP-PrK in vitro and in vivo, yet their functional significance remains unclear. These phosphorylations could, nevertheless, have important roles; for example, they might regulate turnover or alter the ability of these enzymes to interact with other proteins or regulatory molecules.

The most important outstanding problems concern the mechanism of action of hormones such as insulin or epidermal growth factor (EGF) (see refs 5, 136-138 for reviews), that do not appear to use cyclic AMP or Ca²⁺ as second messengers. The interaction of insulin with its receptor in the plasma membrane is known to trigger both increased and decreased phosphorylation of various intracellular proteins, and this hormone is believed to generate a second messenger that regulates a protein kinase(s) and/or a protein phosphatase(s). However, the identity of the second messenger, or the interconverting enzyme(s) that would be its primary target, has remained elusive. It would seem necessary to postulate that an insulin-stimulated protein kinase activates a protein phosphatase, or vice versa, to explain increased and decreased phosphorylation of different proteins by a common mechanism. Alternatively, the insulin second messenger might interact with two or more interconverting enzymes¹³⁹.

A further suggestion is that the insulin-receptor interaction directly activates a protein kinase, perhaps causing its dissociation from the plasma membrane. Each molecule of this protein kinase might then be able to phosphorylate many molecules of each substrate protein, allowing amplification of the hormonal stimulus without the need for any second messenger¹³⁶. The precedent for this idea is EGF. The binding of EGF to its receptor causes the latter protein to be phosphorylated by a protein kinase that is closely associated with the receptor 138 and similar observations have recently been reported for the insulin receptor¹⁴⁰. Curiously, phosphorylation of the EGF receptor occurs on a tyrosine residue. This is most unusual, as phosphotyrosine represents only 0.03% of all phosphorylated amino acids in normal cells, phosphoserine and phosphothreonine accounting for the remaining 99.97% (refs 141, 142). The phosphotyrosine content of cells can be increased up to 10-fold following transformation by some RNA tumour viruses^{141,142} and the transforming gene of several such viruses has turned out to be a tyrosine-specific protein kinase which shows intriguing similarities to the EGF-stimulated protein kinase 138,143-145. These exciting developments implicate abnormal protein phosphorylation as the cause of some virally mediated cancers.

A number of physiological stimuli that do not elevate cyclic AMP, for example, muscarinic-cholinergic stimulators, vasopressin, angiotensin and α -adrenergic stimulation, induce a rapid hydrolysis of phosphatidylinositol in the plasma membranes of their target cells, to yield inositol cyclic phosphate and diacylglycerol. It is therefore of particular interest that a new type of protein kinase has been discovered which has an absolute requirement for diacylglycerol, phosphatidylserine and physiological concentrations of Ca^{2+} (refs 146, 147). This enzyme, termed protein kinase-C, exists in an inactive form in the soluble fraction of all mammalian cells, but in the presence of Ca^{2+} associates with membranes to exhibit full catalytic activity. The Ca^{2+} dependency of this enzyme seems not to be conferred by either calmodulin or related Ca^{2+} -binding pro-

teins. It is attractive to speculate that protein kinase-C mediates the actions of several hormones, with diacylglycerol acting as the second messenger. Further work will be needed to test this hypothesis, although there is some evidence that protein kinase-C is involved in the thrombin-induced release of serotonin from platelets, and a protein of molecular weight 40,000 has been implicated as the major intracellular target for protein kinase-C in this cell^{146,147}.

Finally, I predict that the study of phosphorylation of proteins in the cell nucleus will become a major growth area over the next few years. Protein phosphorylation is very extensive in the

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nucleus, but only in very few cases (histone H1 phosphorylation as a signal for mitosis 148, activation of RNA polymerase I 149) have functional roles started to be defined. I have little doubt that protein phosphorylation will be found to have many crucial roles in the control of gene structure and expression, and progress will surely be facilitated by the advances being made in unravelling the structure of the mammalian chromosome.

The work carried out in my laboratory is currently supported by a Programme Grant from the MRC, and by grants from the British Diabetic Association, British Heart Foundation and Cancer Research Campaign.

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RTICLES

Warm saline bottom water in the ancient ocean

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Considerable isotopic evidence indicates that oceanic bottom water was much warmer in the geological past. A simple convection model driven by multiple turbulent buoyant plumes and observations of present deep water formation have led to the development of a theory for the formation of warm saline bottom water. It is suggested that changes in the size and configuration of marginal seas in net evaporation zones due to lithospheric plate motions and eustatic sea level change caused these seas to become sources of warm saline bottom water. Evidence from the palaeotemperature record and palaeogeography during the late Cretaceous is used to reinforce the hypothesis. Climatic and chemical consequences of the formation of warm saline bottom water are discussed.

THE measurement of oxygen isotope ratios in benthic foraminifera from deep-sea cores has shown that oceanic bottom water has been much warmer in the geological past than it is at present—as warm as 15 °C in the late Cretaceous (about 70 Myr BP). A simple convection model driven by turbulent buoyant plumes developed by Peterson^{2,3} has led us to develop a model for the production of warm saline bottom water (WSBW). We suggest that in the geological past, changes in the size and configuration of marginal seas in net evaporation zones, due to lithospheric plate motions and eustatic (global) sea level changes, caused these seas to become sources of

Turbulent plume model

It has been pointed out that bottom water is formed in small regions. Rossby⁴ suggested that this is because convective buoyancy transfer processes are much more efficient than conductive processes. Thus, to achieve a steady state and hence no net buoyancy flux across the surface, the system evolves so that the convective processes occur on only a small portion of the surface. Moreover, virtually all deep water seems to be formed in semi-enclosed regions (over continental shelves and low- and high-latitude marginal seas)5. We suggest that water trapped in marginal seas is made sufficiently dense, by interaction with the atmosphere, for the outflow to drive a turbulent plume. 6 A characteristic of turbulent plumes is the entrainment of environmental fluid into the plume, an idea which is supported by the observation that, although the Mediterranean water at its source is denser than any water in the Atlantic Ocean, it only penetrates to about 1,200 m. Furthermore, Smith7 esti-

mated that the volume flux of the Mediterranean outflow increases by as much as 10-fold before it terminates at depth and spreads throughout the North Atlantic.

Thermohaline circulation models have tended to treat deepwater formation as a tacit assumption or as a response to global scale forcing. The importance of the outflows of marginal seas as sources of deep water suggested a simple steady-state convection model driven by imposed buoyancy sources. The model is basically the filling of a large tank whose width is very much greater than the plume width scale. The plumes interact with a laterally well mixed interior by the entrainment of interior fluid into the plumes. The model is an extension of one developed by Baines and Turner⁶.

The turbulent plume model is detailed elsewhere^{2,3} but briefly, the model calculations indicate that the depth of termination and horizontal spreading of the plumes is directly related to their buoyancy flux which is the product of the density difference between the plume fluid and the surrounding environment and the volume flux of the plume. The plume with the greatest buoyancy flux will form the bottom water and plumes of lesser buoyancy flux will terminate and spread out at intermediate depths. With two plumes, the termination depth of the weaker plume is relatively insensitive to the difference in buoyancy flux between the two until they become nearly equal in strength. Time-dependent calculations have not yet been made, but it seems that if the plumes switch (that is, the bottom water plume loses buoyancy flux or the intermediate water plume gains buoyancy flux) a relatively rapid and perhaps catastrophic change occurs when the old minor plume becomes the source for the new bottom water.

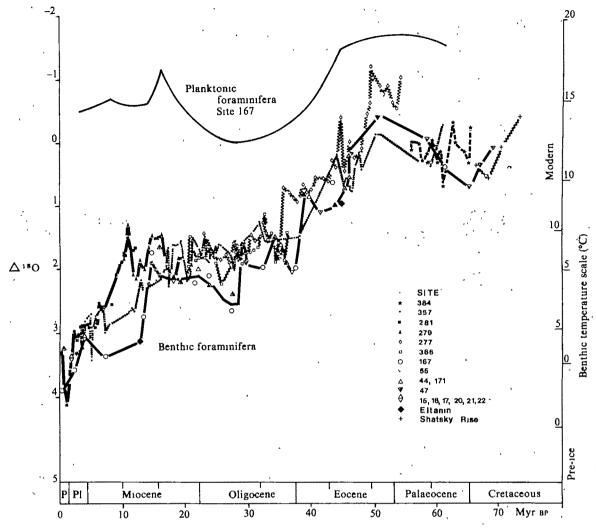


Fig. 1 Oxygen isotope palaeotemperatures from ref. 1.

Discussion

Oxygen isotopic palaeotemperature studies (on benthic foraminifera from deep-sea cores) have shown that the temperature of bottom water has decreased from ~15 °C during the Cretaceous to 3 °C at present (see Fig. 1). Superimposed on the generally decreasing isotopic temperature trend is a series of step changes—some of which are also found in the isotope record of pelagic foraminifera and have been interpreted as shifts in the isotopic composition of the oceans due to sudden increases in ice volumes. After correction for this ice effect, residual oxygen isotope shifts remain in the deep ocean which are not reflected in the surface ocean. These discontinuities in the isotopic record may be due to hiatuses in sedimentation, or they may reflect a series of shorter time scale transitions in bottom water temperature. If these step-wise changes are confirmed, Peterson's competing plume model suggests that deep water is produced by multiple sources, both warm and cold. The temperature and other characteristics of the bottom water are determined by the deep water source area that produces the largest buoyancy flux. These source strengths have varied through time due to changes in areas, location and configuration of marginal seas due to plate motions and eustatic sea level changes. We believe that in the Cretaceous, warm water sources were dominant whereas today cold bottom water is produced at high latitudes. This change necessitated at least one transition from WSBW to cold polar bottom water. However, because of the sensitivity of plume termination depths to small differences in source strengths. several transitions might be expected as a result of variations

in buoyancy fluxes from competing deep-water sources during a period in which the source strengths were nearly the same.

A simple steady-state balance calculation shows that the buoyancy flux from a net-evaporation basin essentially depends on the evaporative flux only and hence the area of the basin and the evaporation rate, and is independent of the salinity. There may be dynamic constraints on the outflow that depend on details such as the configuration of the straits that connect the basin to the ocean; but these constraints are neglected here. We suggest that at times in the geological past, the largest buoyancy flux has emanated from marginal seas in net evaporation zones.

Palaeogeographical considerations

Eustatic sea-level fluctuations can be caused by variations in the global seafloor spreading rate⁹. The area of continent flooded by given increases in sea level is a function of the global hypsography at that time. The size, and hence drainage efficiency, of the continents directly controls the shape of the hypsographic curve¹⁰. Thus, times of increased seafloor spreading and continental breakup generate large areas of flooded continent, termed epicontinental seas, both by raising sea level and by lowering the elevation of the continents.

Epicontinental seas and marginal seas producing WSBW must be located in the zone of net evaporation (10-40 °N and S) associated with the descending branches of the atmospheric Hadley Cell circulation. The distribution of Mesozoic and Cenozoic evaporite deposits strongly suggests that this zone has remained stationary during the past 120 Myr. Figure 2

shows the areas of flooded continents and marginal seas in 10° lat. intervals over the past 100 Myr as measured from the palaeogeographical maps of Barron et al. 11. The most dramatic change during this time has been the decrease in area of shallow and marginal seas in the high evaporation belt $(10-40^{\circ})$.

Because area is an important factor determining the buoyancy flux from net-evaporation basins, the decrease in the area of net-evaporation marginal seas over the past 100 Myr strongly suggests that the late Cretaceous was much more favourable for the production of WSBW. Plots of marginal sea area against time exhibit the same trend as the oxygen isotope temperature record curve from benthic foraminifera. The decrease in area of evaporating marginal seas and the decrease in the temperature of bottom water during the Cenozoic suggests a transition in the mode of deep-water formation in which cooling at high latitudes has played an increasingly important part.

Changes in the latitudinal distribution of epicontinental and marginal seas due to the motions of the lithospheric plates also occur. This movement may transport flooded regions into or out of the net evaporation belt. These movements seem to have had little effect on the areas producing WSBW in the Mesozoic (230-65 Myr BP) and Cenozoic (65-0 Myr BP), but may have been more important in earlier times.

The occurrence of WSBW would have substantial consequences for the ocean and atmosphere. The climatic consequences ^{12,13} of WSBW indicate that the suggested formation of WSBW is consistent with the equable climate of the late Cretaceous. The occurrence of a more equable climate (lesser meridional temperature gradients, with milder conditions at high latitudes) is supported by the occurrence of tropical and temperate faunas and floras, in the terrestrial record, at higher latitudes than at present.

WSBW formation complicates the interpretation of the oxygen isotope record. In the present ocean, dilution with run-off water leads to a decrease in ¹⁸O. The isotopic composition of freshwater entering the northern North Atlantic is approximately -20% and in the Arctic Ocean is about -40% (ref. 14). An understanding of the temperature, salinity and oxygen isotope effects which might be caused by horizontal salinity

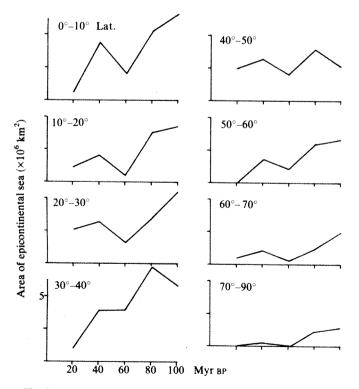


Fig. 2 Area of epicontinental seas plotted against latitude band (from the maps in ref. 11).

gradients can be derived from the consideration of a twodimensional model ocean with a homogeneous deep water containing 90% of the total volume and a surface layer containing 10% of the total. The surface layer is, on average, 5% fresher than the deep water and ranges uniformly from undiluted at the Equator to a 10% diluted at the poles. The diluting freshwater is taken to be -20% the mean salinity and δ^{18} O for the ocean are 35% and 0% respectively. We can calculate the following: the mean δ^{18} O of the surface water is -0.9% ranging from +0.1% at the Equator to -1.9% at the poles. The error in isotopic temperature calculated on the assumption that the ocean is uniformly 0% ranges from -0.4 °C at the Equator to +8.0 °C at the poles. The salinity of the surface water ranges from 35.18% to 31.66% and the deep water salinity is 35.18%. The δ^{18} O in deep water is +0.1%and the temperature error is -0.4 °C. This model assumes, arbitrarily, that the surface water at the Equator has the deep water properties. A 10% reduction in the salinity of polar surface water is sufficient to prevent sinking of polar surface water into deep water at normal salinity and a temperature of 15 °C regardless of the surface water temperature. In practice, the distribution of oxygen isotopes and freshwater in a real ocean are considerably more complicated than this model. In addition to such barriers to deep water flow as oceanic ridges and gateways, climatic and geographical mechanisms may vary the transport of water away from evaporating seas.

Conclusions

Our hypothesis has numerous consequences for the chemistry of the ocean and atmosphere. The chemical state of the ocean when WSBW was dominant would have been quite different from that which exists at present, and the effects should be recorded in the chemistry and isotopic composition of marine sediments. One immediate consequence follows from the fact that the solubilities of many gases strongly depend on temperature and salinity. The increased temperature of WSBW at its source (15 °C compared with -2 °C for present-day cold bottom water) where it is in contact with the atmosphere would reduce concentration of dissolved gases in the source water. Of particular interest are past variations in the concentrations of molecular oxygen and carbon dioxide in deep water as a consequence of WSBW. These variations should leave a signature in the accumulation rates of organic carbon and carbonate in the deep sea.

The distribution of both dissolved oxygen and carbon dioxide within the ocean is the result of exchange with the atmosphere, the formation and destruction of organic matter and physical transport processes. Respiration by animals and bacteria causes a depletion in oxygen in most subsurface ocean waters. The longer a water mass is isolated from the atmosphere the lower its oxygen content becomes; this has enabled oceanographers to use oxygen as a circulation tracer.

Anoxic conditions occur in deep water when the consumption of oxygen by organisms exceeds the rate of oxygen supply. While anoxic conditions prevail organic carbon is accumulated in the underlying sediments at a higher rate. Deep sea anoxic events have been observed in the geological record, as laminated black shales. Some authors 16,17 have explained such events by invoking stagnation resulting from a layer of fresh or brackish surface water or by expansion of the oxygen-minimum layer by inhibited oceanic circulation. These events may also be explained as resulting from a decrease in the ventilation of the deep ocean because of reduced oxygen solubility in the source regions without requiring any change in the circulation rate.

The thermohaline circulation in the ocean is driven by the interaction of the surface ocean with the atmosphere. At low temperatures, the removal of heat is accompanied by an increase in density and the production of deep water. This is the most important source of deep water in the ocean at present. At high temperatures, the addition of heat causes evaporation

and an increase in salinity and density with the same results. We believe this to have been the dominant mechanism for deep water formation in historical times especially in the Cretaceous. The physics and chemistry of the process require further study as do the implications for climate and geology and especially

Received 15 October 1981, accepted 23 February 1982.

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the suggestion that tectonic controls on sea level at various latitudes govern the mechanism of deep water formation.

We thank the NSF for support (grant nos OCE 8111149 and 8024401). We thank Bill Hay, Bill Holser, Erics Barron and Saltzman and Jim Sloan for assistance.

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Enzymatic replication of $E.\ coli$ chromosomal origin is bidirectional

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A soluble enzyme system has been discovered which specifically recognizes and replicates plasmids containing the Escherichia coli chromosomal origin, oriC. Electron microscopy has shown that plasmid replication begins at or near oriC from which it progresses bidirectionally to completion. Control of initiation of a cycle of chromosomal replication and mechanisms of priming and fork movement can now be explored using this system.

REPLICATION of the Escherichia coli K-12 chromosome, as shown by genetic and biochemical analyses in vivo, begins at a unique site (oriC) and proceeds bidirectionally 1-3. The DNA fragment containing oriC has been isolated by its ability to confer autonomous replication on plasmids or phage whose own replication origin has been inactivated⁴⁻⁷. Deletion analysis has localized oriC to a sequence of 232-245 base pairs (bp)8; insertions, deletions or substitutions in this essential region can inactivate oriC.

Initiation of a cycle of chromosomal replication in the cell requires the activities encoded by the genes dnaA, dnaI and dnaP⁹⁻¹¹. In addition, dnaB and dnaC proteins, whose activities are essential for priming the synthesis of nascent chains during replication^{12,13}, are also required during or shortly after initiation^{14,15}. RNA polymerase has also been implicated¹⁶.

A soluble enzyme system has been discovered which specifically recognizes and replicates oriC plasmids 17. The reaction requires dnaA protein, RNA polymerase and numerous replication proteins including dnaB, single-stranded DNAbinding protein and DNA gyrase. Recently, dnaC protein has been shown to be required for in vitro replication of oriC plasmids (unpublished observations). Biochemical analysis of enzymatically synthesized replicative intermediates showed initiation occurring at or near oriC and was consistent with bidirectional progress from that point, but such evidence is essentially a statistical average. Is replication of an individual molecule, once initiated at oriC, then extended bidirectionally to completion? We present here the results of an electron microscopic study which indicate that with few exceptions this enzyme system creates an 'eye' or replication 'bubble' in a plasmid molecule at or near the oriC region which is extended in both directions to generate two complete molecules.

Formation of replicative intermediates

Two classes of supercoiled, oriC template DNA were examined. One, pSY317, is a 13.5-kilobase (kb) plasmid which contains a 5.6-kb EcoRI, oriC fragment (from pSY221)⁵ and a 7.9-kb EcoRI kanamycin-resistance fragment (from pML21)¹⁸. The oriC plasmid contains oriC intact, together with extensive flanking sequences. The second template, M13oriC26, is a 12.2-kb, chimaeric M13 phage DNA containing oriC and its adjacent

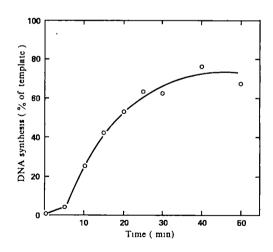


Fig. 1 Time course of oriC plasmid in dnaA-complementing conditions. Reactions were performed as described previously in a 250 µl volume containing 40 mM HEPES pH 7.6, 2 mM ATP, 0.5 mM each of GTP, UTP and CTP, 43 mM creatine phosphate, 100 µM each of dGTP, dATP, dCTP and 5-methyl-³H dTPP (85 c.p.m. per pmol of total deoxynucleotide), 6% (w/v) polyvinyl alcohol 24,000, 11 mM magnesium acetate, 100 μg ml⁻¹ creatine kinase (Sigma), 8.6 μg ml⁻¹ supercoiled pSY317 DNA, 2 mg of protein (fraction II, prepared from E. coli WM433 dnaA204 as described elsewhere 17) and 230 units of dnaAcomplementing activity (fraction III). Incubation was at 30 °C. Aliquots (25 µl) were TCA-precipitated and counted in a liquid scintillation counter. One unit is equal to one pmol of nucleotide incorporated per min.

asnA gene¹⁹. In constructing M13oriC26, the XhoI site immediately to the right of oriC was interrupted. Although interruptions in and around this site do not alter the ability of this and similar oriC plasmids to replicate^{8,19,20}, the directionality of replication was reported to be affected, becoming unidirectional rather than bidirectional^{21,22}.

The extent of replication was limited to obtain a significant number of replicative intermediates for electron microscopic study. The chain terminator, 2',3'-dideoxythymidine 5'-triphosphate (ddTTP) was used. This inhibitor, which prevents chain growth when incorporated into DNA, was present at concentrations relative to dTTP that generated replicative intermediates showing different exents of replication²³. It is assumed that incorporation of a ddTMP residue in the leading or lagging strand at a replication fork stops fork movement. DNAs were purified, spread on parlodion-coated grids by the formamide

c d

Fig. 2 Replicative intermediates of pSY31/ and M13onC26. The reaction with 8.6 μg ml⁻¹ of supercoiled pSY317 was as described in Fig. 1 legend in a 125 µl volume with 50 µM ddTTP, 1,100 µg of E. coli WM433 dnaA204 fraction II and 275 µg of E. coli HB101 (pBF101) fraction II, known to be enriched for dnaA-complementation activity17 Incubation was at 30 °C for 10 min after which reactions were terminated by addition of SDS to 1% and EDTA to 50 mM. Following incubation at 65 °C for 3 min, aliquots were removed for TCA precipitation and counted in a liquid scintillation counter. The sample was then incubated with 0.5 mg ml⁻¹ of proteinase K (Merck) at 37 °C for 60 min, phenol-extracted, etherextracted, and centrifuged in a Beckman airfuge into a CsCl shelf of 1.42 g cm⁻³. The shelf was recovered and DNA was ethanol-precipitated and resuspended in a small volume of 10 mM Tris-HCl pH 8.0 and 1 mM EDTA. Recovery at this step was 60-70% of the initial reaction product. The sample was then incubated with 5 µg ml-1 of pancreatic RNase at 37 °C for 60 min to digest contaminating RNA and either relaxed with SalI endonuclease (given by K. Burtis) in the presence of ethidium bromide31 (a), or restricted to completion with SalI endonuclease in 10 mM Tris-HCl pH 8.0, 10 mM MgSO₄ and 100 mM NaCl at 37 °C for 30 min (b). DNA was then spread on to parlodion-coated grids by the formamide technique24 shadowed at a low angle with platinum-tungsten vapour, and viewed in a Philips 300 electron microscope. Replicative intermediates of M13oriC26 were prepared for electron microscopy as described above except that the sample was either relaxed with EcoRI endonuclease (given by C. Mann) (c) or linearized by digestion to completion with EcoRI endonuclease in 100 mM Tris-HCl pH 7.2, 5 mM MgCl₂, 2 mM 2-mercaptoethanol and 50 mM NaCl at 37 °C for 60 min (d). Scale bars, 0.5 μm.

spreading technique²⁴, and rotary shadowed at a low angle with platinum-tungsten vapour. Replicative intermediates generated with an enzyme fraction (fraction II)¹⁷ from wild-type cells constituted 10–20% of the molecular forms observed, a value consistent with the fraction of template molecules used in

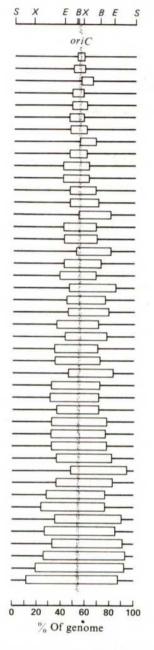


Fig. 3 Replication of the oriC plasmid pSY317 initiates at oriC and progresses bidirectionally. Replicative intermediates generated with 50 µM ddTTP, then purified and linearized with SalI endonuclease (as described in Fig. 2 legend), were randomly selected and photographed. Molecules were analysed with a Hewlett-Packard 9810A calculator by measuring the non-replicated and both replicated segments of an individual molecule on a Hewlett-Packard 9864A digitizer board, averaging the replicated segments, and expressing each portion as a percentage of the total length. Only molecules in which both replicated segments were identical in length (±5%) and for which the total length was within $\pm 10\%$ of the length expected were included in the analysis. Molecules are aligned so that the longer unreplicated segment is to the left, and they are arranged according to increasing extents of replication. The position of oriC is indicated by the stippled area. The open boxes represent the replication 'bubble'. Restriction endonuclease sites are: S, SalI; X, XhoI; E, EcoRI; and B, BamHI.

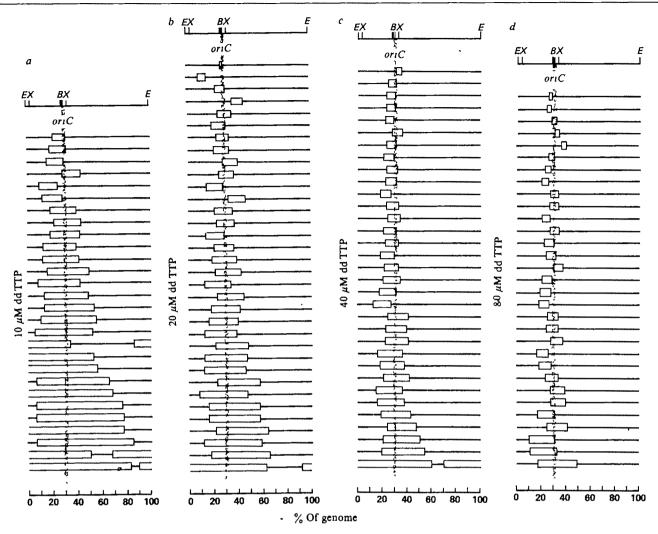


Fig. 4 Replication of M13 oriC26 DNA initiates at oriC and progresses bidirectionally. Reactions were performed as described in Fig. 1 legend in a volume of 125 μl containing ddTTP (as indicated) and 1 mg of enzyme fraction II prepared from E. coli C600 as described elsewhere 17. Incubation was at 30 °C for 15 min. Replicative intermediates produced with a, 10 μM; b, 20 μM; c, 40 μM, and d, 80 μM ddTTP from samples prepared as described in Fig. 2 were restricted, after treatment with pancreatic RNase, with an excess of EcoRI endonuclease, spread on to parlodion-coated grids and examined. Replicative intermediates at each ddTTP concentration were selected randomly, photographed and measured. Only molecules having a correct total length (±10%) in which both replicated segments were the same length (±5%) were included in the analysis. Molecules are aligned so that the longer unreplicated segment is to the right, and they are arranged according to extent of replication. The position of oriC is indicated by the stippled area. The open boxes represent the replication 'bubble'. Restriction endonuclease sites are the same as for Fig. 3.

comparable uninhibited reactions. It is possible that the fortuitous arrangement of a DNA fragment overlapping a circular molecule or unit-length linear fragment can give rise to structures that are indistinguishable from true replicative intermediates, but such events are expected to occur only rarely. Circular replicative intermediates of pSY317 and M13oriC26 appeared in every case as theta-like structures (see Fig. 2).

Defining the initiation site and direction of replication

Replication of an oriC plasmid (for example pSY317) requires dnaA protein¹⁷. Complementation of a crude enzyme fraction (fraction II)¹⁷ prepared from a dnaA mutant provides an assay for purifying dnaA protein overproduced in cells bearing a plasmid carrying the dnaA gene. In a dnaA protein preparation enriched at least 200-fold over wild-type levels, the template molecules were almost completely used in a reaction (Fig. 1).

In conditions which complement dnaA activity, replicative intermediates of pSY317 were accumulated by inhibiting the reaction by 60% with 50 µM ddTTP. To determine the site of initiation and direction of replication, molecules were linearized

by cleavage with SalI endonuclease and examined by electron microscopy (Figs 2, 3). In orienting the molecules in Fig. 3, oriC is located at a point 45% of the genome length from the right end of the linearized pSY317. With few exceptions, the replicated segment of the intermediate overlaps oriC. Although the slight asymmetry of the SalI restriction cleavage relative to oriC introduces some ambiguity in orienting the more extensively replicated intermediates, it is clear that for most of them, replication proceeds bidirectionally. Inspection of the less extensively replicated molecules reveals that initiation occurs at or near oriC. Thus, we have shown that in these conditions, replication of the oriC plasmid pSY317 proceeds bidirectionally from a start in the oriC region.

Replicative intermediates of M13oriC26 DNA were generated with an enzyme fraction (fraction II)¹⁷ from wild-type cells at several levels of ddTTP inhibition and analysed after cleavage with EcoRI endonuclease (Figs 2, 4). In uninhibited reactions, no replication occurred from the M13 origin¹⁷ due to the absence of the M13-encoded gene 2 protein required for replication of supercoiled M13 DNA²⁵⁻²⁷. Inhibition of replication as measured by nucleotide incorporation correlated well with the effect of ddTTP in decreasing the average length of the

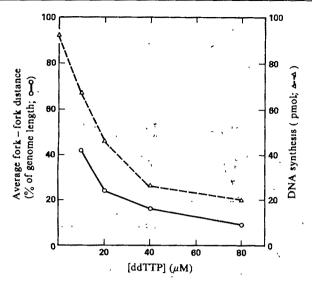


Fig. 5 Inhibition of DNA synthesis by the chain terminator. ddTTP correlates with the fork-to-fork distance of replicative intermediates of M13 or C26. After incubation, aliquots (25 µl) from the reactions described in Fig. 4 legend were removed for TCA precipitation and counted to determine the amount of DNA synthesis. The replicative intermediates of Fig. 4 were then analysed individually for the extent of replication and averaged. Each point represents the average fork-to-fork distance of 28-34 replicative intermediates generated at the corresponding ddTTP con-

replicated segment (Fig. 5). These findings and similar results for pSY317 (data not shown) confirm that the 'eye' and 'Y' forms (molecules with a single fork) are replication intermediates.

The minimal oriC sequence is ~30% of the genome length from one end of M13oriC26 DNA linearized by EcoRI endonuclease (Fig. 4). Recombinant M13 phage DNAs from which oriC has been deleted do not replicate from oriC either in vitro

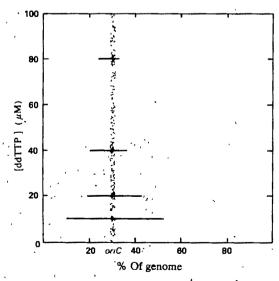


Fig. 6 Replication of M13oriC26 DNA is bidirectional for a population of molecules. Replicative intermediates at each ddTTP concentration shown in Fig. 4 were analysed individually for the extent of replication rightwards and leftwards from oriC. The values were averaged and plotted relative to the position of oriC, indicated by the stippled area, and to the physical map of M13oriC26 linearized at the single EcoRI site. Molecules (4 of 126) which did not seem to have initiated from oriC were excluded from the analysis.

or in vivo 17,28. Molecules aligned in Fig. 4 with the longer replicated segment to the right are consistent with replication initiating from the oriC segment. More extensively replicated molecules clearly indicate that replication proceeds bidirectionally in most cases, and the less extensively replicated molecules show initiation to be at or near oriC.

Replicative intermediates of M13 oriC26 (Fig. 4) were analysed individually with respect to the extent of replication to the right and left of oriC. These values were averaged for each ddTTP concentration and plotted as the distance rightwards or leftwards relative to orlC (Fig. 6). The results indicate that for a population of molecules, replication from ortC is bidirectional.

Of 126 M13oriC26 replicative intermediates examined, 4 were judged not to have been initiated at oriC as the replicated segment did not overlap oriC within the error of measurement (5% of the genome length; Fig. 4). These discrepancies may be due to aberrant initiation or improper breakage of the duplex template. For several molecules of both template DNAs, the bidirectional progress of replication appears asymmetric or even unidirectional, which may be due to asynchrony in the initiation of the two replication forks or in chain terminations by ddTTP. In this regard, examination of the replicative intermediates of phage A and F plasmids, which are known to replicate bidirectionally, also reveals molecules that replicate unidirectionally^{29,30}.

The bidirectional replication of M13oriC26, a template interrupted at the XhoI site, contrasts with the analysis of replicative intermediates of the oriC plasmids²⁰, pOC24, produced in vivo, in which predominantly unidirectional replication was attributed to an interruption at the same XhoI site^{21,22}. This discrepancy may be due to differences in either the plasmid sequences or the experimental conditions; in the in vivo study, replicative intermediates were detected at a frequency of only 1 in 10⁴ plasmid molecules and thus may not represent the principle mode of replication²¹.

This electron microscopic study of individual plasmid molecules replicated in a soluble enzyme system is thus consistent with initiation of replication from oriC. Fork movement from that point progresses bidirectionally. The soluble enzymatic system used here and elsewhere 17 should allow a biochemical approach to the events of priming, fork movement, regulation of initiation and other events occurring at the E. coli origin.

We thank Darrell Dobbertin for assistance in the electron microscopy. This work was supported in part by grants from the NIH and NSF. J.M.K. is a Fellow of the Damon Runyon-Walter Winchell Cancer Fund.

Received 21 December 1981, accepted 8 March 1982

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Linkage of β -thalassaemia mutations and β -globin gene polymorphisms with DNA polymorphisms in human β -globin gene cluster

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Combined analysis of DNA polymorphisms in the human β -globin gene cluster and in cloned β -genes has revealed the association of specific B-thalassaemia mutations and B-gene polymorphisms with particular flanking polymorphisms. A systematic study of cloned genes identified several new mutations, one of which possibly affects transcription. The strategy used may be applicable to other diseases of single-copy genes.

METHODS of recombinant DNA analysis, including restriction endonuclease mapping, gene cloning, DNA sequencing and gene expression assays, now permit detailed study of the molecular basis of human genetic disease. Among the first disorders examined in this context were the β -thalassaemias, inherited conditions affecting synthesis of the β -globin chain of adult haemoglobin^{1,2}. Recently, several types of specific β -globin gene defects have been defined in affected individuals, including deletion of the terminal end of the gene in Asian Indians³⁻⁵, chain termination (or nonsense) mutations in Chinese and Mediterraneans⁶⁻⁹, a frameshift deletion in a Turkish patient⁷, a point mutation within an intervening sequence (IVS) in Mediterraneans^{10,11}, and an IVS splice junction mutation in some Middle East individuals 12 and a Mediterranean (R. H. Treisman, N. Proudfoot, M. Shander and T. Maniatis, in preparation). The disparate β -thalassaemia genes analysed so far have been largely unselected or at best chosen for study on the basis of their phenotype, either β^0 or β^+ , corresponding to absent or reduced β -globin production. Using this rather random approach, the full spectrum of DNA lesions responsible for a genetic disorder may not be apparent, as cloning of mutant genes from individuals having similar phenotypes is naturally biased towards the study of the most prevalent alleles. Without using a directed approach for selection of genes for further examination, the search for new, and potentially informative, mutations is encumbered by repeated analysis of previously characterized genes.

In this study, we have developed and applied a new strategy for the comprehensive analysis of existing mutations in a class of human disease. Specifically, we have combined analysis of various restriction enzyme polymorphisms in the β -globin gene cluster with direct examination of β -globin structural genes in Mediterranean individuals having β -thalassaemia. The basis of

this approach is our finding that specific mutant genes are strongly linked to patterns of restriction site polymorphism (haplotypes) in this region of the genome. Systematic characterization of genes representing all haplotypes in an extensive panel has revealed several new mutations and the nature of sequence polymorphisms within the β -globin gene itself. This approach to the study of β -thalassaemia should be readily applicable to the systematic analysis of specific mutations of other single-copy genes which result in human disease.

Restriction site polymorphisms in β -thalassaemia DNA

Several restriction site polymorphisms distributed over more than 60 kilobases (kb) of DNA within the human β -globin gene cluster have been described ¹³⁻¹⁷. In an attempt to characterize in detail the DNA regions in which mutant β -globin genes lie, we have assessed cleavage at seven of these polymorphic sites in a panel of 91 B-thalassaemia chromosomes of Mediterranean origin. Nine different cleavage patterns, one of which was found along each chromosome, were observed and designated haplotypes I-IX (Fig. 1). Individual haplotypes accounted for 1-47% of the thalassaemia chromosomes studied. When analysing the same sites in normal DNA samples, we observed non-random association of the five sites located 5' to the Bglobin gene (HincII and HindIII sites) and non-random association of the two sites located 3' to the 8-globin gene (the AvaII and BamHI sites)16. However, the 5' and 3' collections of sites appeared to associate independently to generate the various haplotypes¹⁶. In general, the polymorphic patterns in the thalassaemic DNA samples did not differ in frequency from patterns seen in normal individuals.

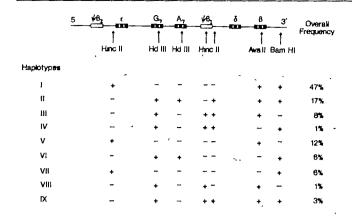


Fig. 1 Chromosome haplotypes in β -thalassaemia. Polymorphic restriction enzyme sites in the β -globin gene cluster that were used here are shown at the top in a schematic representation of the β -gene cluster. A total of 91 chromosomes of Mediterraneans with β -thalassaemia were examined at these sites by Southern³⁴ gel blotting, using methods and probes described elsewhere¹⁶. Patients were of both β^0 and β^+ phenotypes. + Indicates the presence of cleavage at a particular site, and – indicates absence of cleavage. Haplotypes indicate the patterns of cleavage along a chromosome and are arbitrarily numbered I-IX. Hd III, Hin dIII. ε , G_{γ} , A_{γ} , δ and β are the expressed genes of the cluster δ^{35} . $\psi\beta_2$ and $\psi\beta_1$ are pseudogenes identified by gene cloning δ^{35} .

On the one hand, haplotypes might reflect the presence of specific β -thalassaemia alleles in a population, in which case they would mirror the chromosomal background on which a mutation arose. On the other, several different thalassaemia mutations might be regularly found in each chromosome type, indicating no relationship between these haplotypes and specific defects.

Coupling of specific β -thalassaemia defects to specific haplotypes

In a first attempt to characterize specific mutations in β -thalassaemia, we examined randomly chosen DNA samples from patients clinically homozygous for β^0 -thalassaemia. β -Globin genes were cloned with their flanking sequences as a 7.5-kb *Hind*III fragment in bacteriophage Charon 28 (ref. 18), as previously described⁷. Surprisingly, our first three β -gene clones (one of Greek and two of Italian origin) all showed the same nucleotide substitution, within codon 39, yielding a translation stop signal 6-9 (see Table 1). Examination of the polymorphic restriction sites in the β -gene cluster of these three patients and their parents revealed linkage of the nonsense mutation genes with haplotype II chromosomes.

The coupling of one haplotype with a specific thalassaemia mutation suggested that each haplotype might indicate the presence of a specific mutant allele. Isolation and characterization of β -globin genes from thalassaemia DNAs representing the various haplotypes in our panel might then provide an efficient, systematic approach to the complete molecular description of the disorder in this population. We chose DNA samples that would permit rapid, unequivocal coupling of haplotypes with DNA mutations within β -genes. We did this by selecting patients homozygous for specific haplotypes, when available, or those heterozygous at the AvaII site located in the second intervening sequence (IVS-2)¹⁶⁻¹⁹ of the β -gene. In the latter case, by analysing bacteriophage clones containing the β -genes, we deduced which of the two β -genes had been cloned, even though the cloned region did not contain any of the other polymorphic sites used to define the haplotypes.

Further striking evidence of coupling of haplotypes and specific mutant genes was obtained for two other previously

described β -thalassaemia genes. First, three β -gene clones arising from haplotype I chromosomes contained the $G \rightarrow A$ substitution within IVS-1^{10,11} that leads to aberrant processing of precursor β -RNA²⁰ and a β ⁺ phenotype (Table 1). Second, a gene isolated from a haplotype III chromosome of a Turkish individual revealed a $G \rightarrow A$ transition at the splice junction of IVS-2 (Table 1), a mutation that prevents normal RNA processing (R. H. Treisman, N. Proudfoot, M. Shander and T. Maniatis, in preparation) and yields a β ⁰ phenotype 12. Because this mutation alters a cleavage site for the restriction enzyme HphI normally found at the IVS-2 splice junction 12,19, we analysed our DNA samples by restriction mapping with this enzyme. All of six other chromosomes of the haplotype III pattern tested demonstrated the abnormal gene map after HphI digestion,

Table 1 Summary of DNA sequences of β -thalassaemia genes Other sequence changes Haplotype Thalassaemic mutation IVS-1 None TTAGTCTATTTTCCACCTTAGGCTG, Exon-2 38 39 П None GIn (C) Arg Stop III IVS-2 aplice junction G→T (position 74, IVS-2) Exon-1 G→T (position 74, IVS-2) splice junction GIY (G) GGCAGATTG IVS-1 consensus ٧ı GIV ATO GGCAGGTTGGCATC IVS-2 (internally) CAGGTACCAT 5 flanking region G→T (position 74, IVS-2) VIII CCACACGCTAGGGTTGGCCAATCT IX

β-Globin genes were cloned in 7.5-kb HindIII restriction fragments of genomic DNA in bacteriophage Charon 28 (ref. 18). Genomic DNA was digested to completion with HindIII and centrifuged in a 10-47% sucrose gradient in a Beckman SW27 rotor at 27,000g for 23 h. DNA of ~7.5 kb was ligated to calf alkaline phosphatase-treated HindIII arms of Charon 28 DNA and packaged in vitro into viable phage³ Phage were screened by the Benton-Davis procedure³¹ using probe prepared by nick-translation from a plasmid containing the BamHI-EcoRI fragment encompassing IVS-2 of the normal β -gene (provided by Arthur Nienhuis). The cloned β -globin genes of positive recombinants were subcloned as 4.4-kb Pst fragments in pBR322 for DNA sequence analysis. All DNA sequencing was performed by the method of Maxam and Gilbert³². Fragments were labelled at their 5' or 3' termini and either strand-separated or subjected to secondary digests before sequencing. We analysed 13 independent β -globin genes of 10 unrelated β -thalassaemic patients. Genes of haplotypes V-VIII were sequenced completely; those of haplotypes I-IV and IX were extensively sequenced, except for the central 500 bp of IVS-2. Representative examples of genes of haplotypes I-III have been sequenced completely by others (refs. 8, 10, 11 and R. H. Treisman et al., in preparation). Normal sequences are shown in parentheses above the putative thalassaemic mutations. Haplotype VI-VIII genes are separated from the others to indicate that the proposed mutations require further supportive evidence from functional studies (see text).

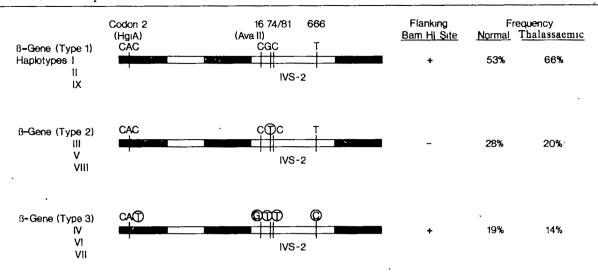


Fig. 2 β -Globin gene frameworks (gene polymorphisms). Schematic representation of polymorphic DNA sequences detected in β -globin gene clones (see Table 1). Except for the single nucleotide substitutions that lead to thalassaemia, the β -genes of haplotypes I, II and IX are identical in sequence to the normal gene sequenced by Lawn et al. ¹⁹. Nucleotide sequence differences circled in the type 2 and type 3 genes are polymorphisms on the basis of their presence in multiple β -globin genes with different primary defects (see Table 1) and by the presence of all five changes of the type 3 gene in a cloned AvaII(-) gene of a non-thalassaemic Greek individual. Type 3 genes differ from type 1 and type, 2 genes in lacking two intragenic restriction sites: the CAC \rightarrow CAT change in codon 2 obliterates an HgiA I site and the $C \rightarrow G$ substitution 16 nucleotides into IVS-2 alters an AvaII site ¹⁶.

whereas 34 of 36 β -thalassaemia chromosomes of various other haplotypes did not (see Table 2 and below). Therefore, this specific mutation was strongly coupled to haplotype III chromosomes on the basis of both gene cloning and restriction mapping.

In view of the coupling of three specific β -thalassaemia genes with haplotypes I, II and III, we analysed single genes representing the remaining haplotypes to elucidate the spectrum of mutant genes in this population. As indicated below, when possible, we assessed by restriction mapping the coupling of additional, new mutant genes with haplotypes.

DNA sequences of cloned β -genes

We examined the DNA sequence of 13 independent β -globin genes of 10 unrelated β -thalassaemia individuals, and a normal gene with the AvaII restriction polymorphism in IVS-2. Each previously undescribed mutant gene has been entirely sequenced. It is noteworthy that eight different β -thalassaemia genes were represented in the clones encompassing all nine haplotypes in our panel. Genes isolated from haplotypes IV-VIII revealed new mutations (see Table 1). Several conclusions regarding normal β -gene sequences and new putative thalassaemia mutations are derived from these data.

β-Globin gene polymorphisms: When compared with the normal gene sequence reported by Lawn *et al.*¹⁹, nucleotide substitutions that were common among different β-thalassaemia genes identified five polymorphisms of the β-sequence: a $C \rightarrow T$ substitution within codon 2, and $C \rightarrow G$, $G \rightarrow T$, $C \rightarrow T$ and $T \rightarrow C$ changes in IVS-2 positions 16, 74, 81 and 666, respectively. That these five changes are true polymorphisms was démonstrated by examining a normal gene of the $Av\Pi(-)$ variety.

Further consideration of these polymorphisms indicated the existence of three β -globin gene frameworks into which various thalassaemia mutations have been introduced (Fig. 2 and Table 1). Type 1 is characteristic of β -genes of haplotypes I, II and IX and contains no DNA polymorphisms. Type 2 genes have a single polymorphism, the $G \rightarrow T$ substitution at IVS-2 position 74, and include thalassaemic genes of haplotypes III, V and VIII. Type 3 genes, encompassing haplotypes IV, VI and VII,

display all five polymorphisms. These various gene frameworks can be identified in the Mediterranean population by restriction mapping in that type 2 genes are linked to a BamHI polymorphism. So to the β -gene (see Fig. 1 and Table 1) and type 3 genes are recognized by the IVS-2 AvaII polymorphism. By restriction mapping, we estimate that type 1, 2 and 3 genes, respectively, represent 53, 28 and 19% of β ^-genes and 66, 20 and 14% of β -thalassaemia genes in the Mediterranean population (Fig. 2).

Putative thalassaemia mutations: From their DNA sequences, we have identified eight different β -thalassaemia alleles (Table 1), four of which have been reported previously. The molecular defects of genes isolated from haplotypes I-IV are either evident from the nucleotide sequence itself⁷⁻⁹ or established by gene expression in heterologous systems (ref. 20 and R. H. Treisman, N. Proudfoot, M. Shander and T. Maniatis, in preparation). Of the new mutant genes isolated here, that present in haplotype V, which contains a G -> A substitution within the IVS-1 splice junction, is obviously a mutant of RNA processing, analogous to the IVS-2 splice junction mutant (R. H. Treisman et al., in preparation). The two genes derived from haplotype VI and VII chromosomes are also possible mutants of β -RNA processing. In particular, the single substitution within the conserved consensus sequence²¹ of the IVS-1 splice junction in the gene of haplotype VI (Table 1) may have an adverse effect on either the rate or fidelity of processing of the RNA precursor. The $C \rightarrow G$ substitution at IVS-2 position 745 in the gene of haplotype VII (Table 1) generates a new CAGGT sequence that could well serve as a new (and deleterious) internal splice site²¹ in the β -RNA precursor. The accumulation of large amounts of β -RNA precursor containing IVS-2 sequences in the erythroid cells of a β^+ -thalassaemia individual with this gene is consistent with this nucleotide change (T-C. Cheng and H.H.K., unpublished observations).

The gene isolated from haplotype VIII is a novel β -thalassaemia allele that is an excellent candidate for the first naturally occurring mutant of gene transcription in a mammalian system. Except for the IVS-2 position 74 polymorphism, its sequence is identical to that of the normal gene reported by Lawn et al. ¹⁹. Upstream from the start of the β -gene at position -87, however, a single substitution $(C \rightarrow G)$ is observed in the vicinity of the CCAAT homology²², a region which seems

Table 2 Linkage of haplotypes with β -thalassaemia mutations analysed by restriction enzyme analysis

Defect and associated haplotype	Restriction enzyme used	β-thalassaemia alleles of the specified haplotype that were positive/no. tested	β-thalassaemia alleles of other haplotypes that were positive/no. tested	β^{A} -alleles that were positive/no. tested
IVS-2 splice (III)	HphI	7/7	2/36	0/27
IVS-2 position 745(VII)	RsaI	5/6	0/49	0/42
-87 substitution (VIII)	AvrII	1/1	0/6	0/16

The presence of the specific defects was assessed by restriction mapping. For analysis of the IVS-2 splice junction mutation, the labelled probe was an EcoRI-BamHI fragment that spans IVS-2: the normal β -gene fragment is 1.1 kb and the mutant, 1.4 kb. DNA digested with RsaI was analysed with β -cDNA³³ as probe. The creation of a new RsaI site by the IVS-2 position 745 substitution reduces the length of a normal 2.2-kb fragment to 1.9 kb. A 5' β -gene probe, extending from the extragenic HpaI site to the intragenic BamHI site, was used to analyse DNAs doubly digested with AvrII and HindIII. In this case, the normal $5'\beta$ -fragment is 2.3 kb, while the mutant band is \sim 4 kb. Values are the number of alleles that were positive for the thalassaemia defect as detected by the restriction enzyme, divided by the number of alleles tested, either within a haplotype, outside it, or in the normal Mediterranean population.

Table 3 Gene incidence based on polymorphism haplotype Total no. with haplotype Haplotype Thalassaemia defect identified No. with defect/no. tested Italians Turks IVS-1 B+ ... 4/4 14 (31%) 28 (67%) п Nonsense codon 39 3/3 11 (24%) 3 (7%) 5' IVS-2 splice Ш 7/7 · 3 (7%) 2 (5%) Frameshift codon 8 1/1 5' IVS-1 splice 1/1 4 (9%) 7 (17%) 5' IVS-2 splice 2/9 VI 5 (11%) 1 (2%) IVS-1 consensus substitution 1/1 VII IVS-2 position 745 5/6 5 (11%) 1(2%)1 (2%) VIII -87 substitution 1/1 IX Nonsense codon 39 1/1 2 (5%) 26/34 45 (100%) 42 (100%) Total - 91 genes

The presence of specific defects was assessed by gene cloning and DNA sequencing (Table 1) and by restriction enzyme digestions using HphI, RsaI and AvrII (see Table 2 legend). Although we have cloned and sequenced only 3 genes of haplotype I, the β^+ -thalassaemia patient studied by Spritz et al. (whose DNA was given to us by B. G. Forget) was homozygous for this haplotype and is scored as one allele here. Of 34 β -thalassaemia genes that were analysed directly, 26 have been assigned a specific mutation. Within haplotype V, HphI digestion excluded the IVS-2 splice junction mutation in seven genes. If direct detection of the IVS-1 splice junction mutation in uncloned DNA were possible, as many as 33 β -thalassaemia genes out of 34 assayed might have been linked to a specific defect.

important in the efficient initiation of gene transcription^{23,24}. Functional studies of these new thalassaemia genes are in progress to establish unequivocably whether the various nucleotide changes result in the β -thalassaemia phenotype.

Although the mutations resulting in thalassaemia in the genes of haplotypes VII and VIII have not been rigorously established by functional criteria, additional restriction mapping data provide further support for the particular mutant assignments and also for coupling of these defects with their respective haplotypes. The IVS-2 position 745 substitution in the haplotype VII gene creates a new RsaI restriction site (GTAC)²⁵. Restriction mapping of both thalassaemic and normal DNAs with RsaI has been used to investigate whether this nucleotide change is a normal polymorphism and whether it is limited to haplotype VII thalassaemia chromosomes. As shown in Table 2, an abnormal RsaI map was found for five of six genes present in haplotype VII chromosomes, but in no other normal (β^{A}) or β -thalassaemia genes. The observed agreement suggests strongly that this single substitution is the primary mutation and is linked only to haplotype VII β-thalassaemia chromosomes. An additional nucleotide substitution within the 5' untranslated region of this gene (Table 1) is probably a DNA polymorphism that may subdivide framework 3 genes.

Similarly, the substitution upstream from the β -thalassaemia gene of haplotype VIII alters a normal AvII restriction site. An abnormal AvII restriction map was found in uncloned β -thalassaemia DNA of haplotype VIII but neither in β -thalassaemia genes of other haplotypes nor in β ^-DNA (Table 2).

Exceptions to simple coupling of haplotypes with specific defects

While the simplest situation would be the absolute linkage of each haplotype to a different mutation, we have found that this is not the case. We have observed two types of exception: (1) the association of a single mutation with more than one haplotype; and (2) the presence of more than one mutation within a single haplotype.

The codon 39 nonsense mutation was identified in a clone having the rare haplotype IX as well as in three clones having the common haplotype II (Table 1). The second type of exception was observed in genes of haplotype VII. As noted above, one of six genes of this haplotype had a normal RsaI restriction map, indicating the presence of another type of β -thalassaemia gene in this group. Study of the IVS-2 splice junction mutation by HphI restriction mapping revealed both types of exception. Although all of seven genes of haplotype III have this mutation, two of nine genes of haplotype V that were tested also showed this defect, whereas all of 27 β -thalassaemia genes of other haplotypes did not (Tables 2, 3). From these data, we suggest that the IVS-2 splice junction mutant comprises a minority of haplotype V B-thalassaemia genes, while the IVS-1 splice junction mutant constitutes the majority. Although the presence of the nonsense codon 39 and IVS-2 splice junction mutants in two different haplotypes is consistent with multiple, independent origins of these mutations, recombination between $\psi\beta_1$ and β -genes is a more likely alternative in view of the independent assortment of the 5' and 3' collections of restriction sites

in the β -gene cluster¹⁶. Only the finding of identical mutations in β -genes of different basic frameworks would provide clearcut evidence for multiple origins of a specific mutation.

Frequency of specific mutations and genetic heterogeneity

By combining gene cloning with restriction analysis, we have determined the specific mutation in 26 β -thalassaemia genes (Table 3). Of 20 genes associated with the haplotypes I-III and VII, 19 displayed linkage of mutation to haplotype. These four haplotypes are associated with 81% of Greek and 73% of Italian β -thalassaemia alleles. This strong relationship allows us to estimate the incidence of the various alleles in these Mediterraneans, as shown in Table 3. Molecular heterogeneity of β -thalassaemia is so extensive that only a minority of patients are homozygous for any defect. 26 of 30 Italian and 11 of 22 Greek patients were genetic compounds and not homozygotes. Haplotypes for β -thalassaemia appear to associate randomly, as the expected numbers of genetic compounds were 81% and 48% for Italians and Greeks, respectively, while 86% and 50% were observed.

Conclusions

The β -thalassaemias are among the first human genetic diseases to be examined using new techniques of recombinant DNA analysis. Previous studies used essentially unselected samples for molecular cloning and gene analysis. Genes of affected individuals were generally chosen on the basis of a clinical phenotype, that is β^+ or β^0 -thalassaemia, with little attention being paid as to whether they were likely to differ. The existence of multiple restriction site polymorphisms within the β -globin gene cluster allows the detailed characterization of chromosome regions in which mutant β -globin genes reside. Taking advantage of these polymorphisms to subdivide a panel of β thalassaemia genes, we have isolated eight different mutant genes among the nine different haplotypes represented in Mediterraneans. Repeated isolation of the same mutant gene was largely avoided by selecting β -genes on the basis of their associated haplotypes. Seven of these eight genes are present in Italians from various locales in Italy and six are present in Greeks. Our results suggest that only rare additional alleles are likely to be discovered among Mediterraneans in further studies.

The coupling of specific mutations and haplotypes is an example of linkage disequilibrium in which specific alleles become associated with neutral changes in the DNA. Disruption of these linkages could result from multiple origins of specific defects or DNA recombination (or mutation) that would alter the associated restriction site polymorphisms. As indicated above, we have found examples of discordance of single defects with haplotypes, which may have arisen by recombination.

When specimens of different racial backgrounds are examined, we find that the mutations identified in Mediterraneans are not present in chromosomes of the same haplotypes. For example, Asian Indians with haplotype III and VII chromosomes did not have the mutations recognized by restriction mapping with either HphI or RsaI (unpublished observations), although almost all samples from Mediterraneans did. Therefore, similar analyses of the kind described here may uncover new sets of mutant genes in other populations.

Our analysis of cloned genes delineates the nature of sequence polymorphism within the β -gene itself. Rather than observing randomly scattered sequence polymorphisms, we found three gene frameworks (Fig. 2) into which mutations were introduced. The presence of a common polymorphism (that at IVS-2 position 74) in frameworks 2 and 3 suggests that it may be possible to develop an evolutionary history of β -genes by analysing additional clones. The presence of these frameworks in β -thalassaemia genes, as well as coupling of specific mutations to haplotypes, suggests that the observed mutations are of relatively recent origin. We conclude that they arose after fixation of the haplotypes and β -gene frameworks.

Our data indicate that only 15 of 87 (\sim 17%) mutant genes in Italians and Greeks can be detected directly by restriction enzyme analysis in uncloned DNA using HphI, RsaI or AvrII (Table 3). Thus, direct DNA analysis of β -thalassaemia mutations will have a small impact on prenatal diagnosis in Mediterraneans unless new enzymes are discovered that recognise the common defects. DNA polymorphism analysis, on the other hand, is highly useful for prenatal diagnosis when linkage analysis within a family can be carried out 26,27. Such linkage analysis is necessary because similar haplotype patterns are associated with normal and β -thalassaemic chromosomes. The situation in β -thalassaemia is in contrast to that in sickle cell anaemia which can now be detected directly in DNA using either DdeI or MstII28,29

The coupling of haplotypes and specific mutations of a singlecopy locus within a defined population is probably a general finding as it reflects the origin of rare mutations in particular chromosomal backgrounds. We anticipate that the strategy used here will prove useful in characterizing genes associated with other inherited diseases and in further study of globin mutants. This approach should greatly facilitate the search for novel mutations among phenotypically similar individuals and the complete molecular description of a genetic disease.

This research was supported by grants from the National Foundation-March of Dimes and the NIH to S.H.O. and H.H.K. and the Cooley's Anemia Fund to H.H.K. We thank Eric Rosenvold of New England BioLabs for a gift of the restriction enzyme AvrII; David G. Nathan and John A. Phillips for critical comments; and the many physicians who have referred to us for prenatal diagnosis testing families at risk for β-thalassaemia. We thank C.N. Lewis for preparing the manuscript.

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LETTERS TO NATURE

A new thermometer for external galaxies

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We introduce a new spectroscopic method at radio frequencies to measure the temperature of giant cloud complexes in external galaxies. We have detected the $\lambda=1.3$ cm J,K=2,2 inversion transition of NH₃ in the nucleus of the galaxy IC342. This result, taken together with our previous detection of the J,K=1,1 transition allows us to deduce a kinetic temperature of 50 ± 15 K and an equivalent size scale of at least 25 pc for the molecular gas. The results are consistent with line formation in the nucleus where very active star formation is taking place.

Microwave observations of the spectral lines of various molecules have been used with varying degrees of success to derive density, temperature, size scale and mass of clouds in the interstellar medium²⁻⁴. To infer gas kinetic temperatures for molecular clouds within our own Galaxy, the lower rotational transitions (J = 1-0, J = 2-1, J = 3-2) of CO and the inversion transitions of the lower metastable states (J = K) of NH₃ have proven to be most reliable. The CO transitions (mainly J = 1-0 observations) appear to be optically thick and to be in thermodynamic equilibrium with H₂ through collisions. Thus, the brightness temperature of the line reflects the kinetic temperature (T_{K}) of the molecular cloud. The technique with NH3 utilizes the fact that the metastable states are coupled to each other principally through collisions with H2 because the radiative lifetimes between the K-ladders are very slow. Therefore one can derive from the ratios of population in several nearby metastable states a rotational temperature (T_R) which in fact closely approximates T_K . Most commonly, the J, K = 1, 1and 2, 2 inversion transitions are used with the assumption that these lines are approximately optically thin, as indicated by the relative intensities of hyperfine structures. As these two metastable doublets are close in frequency, their excitation will be similar. The ratio of antenna temperature is then (see ref. 5, for basic formulae)

$$\begin{split} \frac{T_{\rm A}(2,2)}{T_{\rm A}(1,1)} &\cong \frac{\tau(2,2)}{\tau(1,1)} \cong \frac{\nu_{2,2}|\mu_{2,2}|^2 N_{2,2}}{\nu_{1,1}|\mu_{1,1}|^2 N_{1,1}} \\ &\cong \frac{20}{9} f \, {\rm e}^{-41 \, 5/T_{\rm R}} \end{split}$$

$$N_{JK} \propto g_{JK} (2J+1) \exp(-h(BJ(J+1)+(A-B)K^2)/kT_R)$$

 $|\mu_{JK}|^2 = K^2 \mu_0^2/J(J+1)$

where τ is the optical depth, N is the column density, μ_0 is the dipole moment, g_{JK} is a statistical factor accounting for the nuclear spin of the hydrogens $(g_{JK} = 1 \text{ for } K \neq 3n, g_{JK} = 2 \text{ for } K = 3n)$, A and B the rotational constants, and f accounts for whether or not the hyperfine structure is resolved (f = 1 if unresolved).

Observationally, the ratio of the J, K = 1, 1 and 2, 2 line strengths is quite sensitive to temperature in the range 5-80 K. The empirical results show that the ratio of $T_A(1, 1)/T_A(2, 2)$ is reliable in distinguishing between regions of various temperatures. For example, $T_A(1, 1)/T_A(2, 2) \approx 1$ for H II regionlarge molecular clouds ($T_K = 30-70$ K), and $T_A(1, 1)/T_A(2, 2) \approx 10$ for dark clouds ($T_K = 10$ K). Additionally, these two transitions (at $\nu_0 = 23.7$ GHz) are separated by only 28 MHz in frequency so that they can be measured simultaneously with the same receiver and the same spectrometer. Thus, their ratio

is well determined independent of the absolute calibration of the spectra.

A comparison of the observational results of these two techniques using CO and NH₃ is shown in Fig. 1 for various types of interstellar clouds in the Galaxy. The correlation between the two methods indicates that these independent techniques are both sampling the gas temperatures. The advantage of the NH₃ observational method is that it circumvents the following difficulties, especially in extragalactic studies. First, beam dilution: the CO observations must spatially resolve the source to determine the brightness temperature reliably. In the Galaxy, interstellar CO observations generally resolve the source. However, for the external galaxies, the CO data suffer from an indeterminate amount of beam dilution. In contrast, the analysis based on NH₃ data depends on the ratio of line strengths. Because the NH₃ optical depths are in general low and the excitation for the two lines are comparable, any beam dilution effects are cancelled out in taking a ratio. Second, self absorption: several CO lines in galactic sources exhibit self absorption features due to temperature and/or density gradients in the cloud combined with the high opacity of the CO transition. Again because of the low opacities of the NH3 lines, selfabsorption effects are not observed and are not likely to be important. For the external galaxies, we therefore recognize that studies of the NH₃ molecule represent an important new

Improvements in sensitivity have allowed radio astronomers to begin to use molecular line observations to study the external galaxies⁶. CO exhibits the strongest lines and has been the most fruitful molecule for study so far. For the galaxies where the CO line is most intense, the emission has been partially mapped and serves as a tracer of the H_2 density. Thus, in combination with H I data, the total hydrogen distribution can be studied in galaxies. However, without a priori knowledge of the spatial

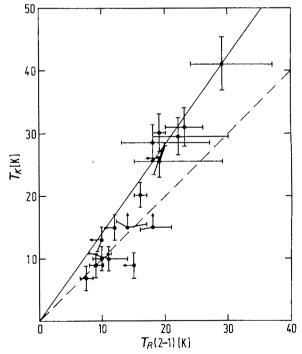


Fig. 1 A comparison of $T_R(2-1)$ as deduced from NH₃ and T_K as deduced from CO for various sources in the Galaxy^{8,9}. The dashed line is $T_R(2-1) = T_K$ and the solid line is $T_R(2-1) = 0.7 T_K$ (empirical results for compact H II regions⁸). The correlation demonstrates the reliability of temperatures derived through NH₃ measurements as compared with CO measurements. The exact coefficient is uncertain but theoretical calculations indicate that in general $T_R(2-1)$ will be less than T_K .

structures, CO cannot be used to measure gas temperatures in this instance due to the severe beam dilution. The detection of extraglactic NH₃ through its two lowest matastable transitions therefore constitutes indeed a new thermometer for giant gas complexes in external galaxies.

Using the MPIfR 100-m telescope, we have now detected the J, K = 2, 2 inversion transition of NH₃ in the nucleus of the ScD galaxy IC342, in addition to our previous detection of the J, K = 1, 1 transition¹. We can thus derive the first reliable measure of the kinetic temperature for extragalactic molecular gas. The observations were made using a cooled parametric amplifier frontend ($T_{\text{sys}} = 200 \text{ K}$) and a 256×312.5 kHz filterbank spectrometer. Both the J, K = 1, 1 and 2, 2 lines fall within the passband of the spectrometer. We detect a main beam brightness temperature of 0.032 ± 0.004 K for the J, K = 1, 1transition and 0.030 ± 0.004 K for the 2, 2 transition. Taking into account the errors in the measurements and the uncertainty in the conversion from T_R to T_K , we estimate the kinetic temperature to be 35-70 K. A detailed analysis of these results is given elsewhere7; we briefly summarize our findings as follows. From the detected brightness temperatures, we infer that the emission must cover an equivalent area with a diameter of

Received 3 December 1981; accepted 19 February 1982

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at least 25 pc. From excitation arguments, we derive excitation temperatures and hence densities. We then estimate the total mass as a function of size scale. Together with estimates based on the column density and abundance arguments, we arrive at a total mass detectable via the NH₃ lines of the order of $10^7 M_{\odot}$. Such large and warm gas complexes imply a large cooling rate due to emission in atomic and molecular lines. A possible heating mechanism would be warm dust grains which must first be heated by direct star light. We therefore infer the presence of numerous young OB stars. Detailed calculations indicate that 103-105 OB stars would be required in the nuclear region of IC342. We conclude that the detection of the NH3 lines, indicating the presence of warm gas, implies very active star formation in the core of this spiral galaxy. In fact, the star formation rate may be 10 times higher than within the nuclear region of our own Galaxy.

In spite of its weak lines, NH3 may be the most promising molecule, for investigations of the physical conditions of molecular clouds in galaxies.

P.T.P.H. acknowledges support from the Miller Institute for basic research and NSF grant AST78-21037. We thank T. L. Wilson for helpful comments.

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Observing interplanetary disturbances from the ground

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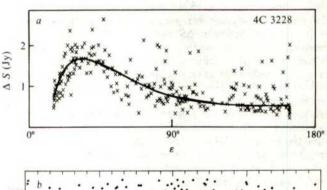
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The scintillation of celestial radio sources due to small-scale turbulence along lines of sight through the interplanetary medium provides a convenient, ground-based method of monitoring disturbances in interplanetary space. With the sensitive 3.6-hectare Array at Cambridge we have carried out a new programme in which ~900 sources were observed each day for more than 1 yr. When the long-term average scintillation behaviour of each source had been accurately determined we found that transient disturbances could be clearly distinguished. We detected large clouds of enhanced turbulence moving out from the Sun and were able to track them to distances beyond the Earth's orbit. Our observations differ from recent work elsewhere in that we used a larger grid of sources and observed them more continuously over a wider range of solar elongations. To illustrate the information that is now being obtained we present observations for 10-21 December 1978, a period of considerable solar activity for which spacecraft and other data are also available.

The measurements were carried out at 81.5 MHz with the 3.6-hectare Array which enables sources to be observed simultaneously at meridian transit over a wide range of declinations2. Intensity scintillation in the band 0.1-3 Hz was detected, averaged and sampled at intervals of 10 s on to magnetic tapes. The noise fluctuation per sample was ~ 0.5 Jy and each source was observed for ~120 s. Data contaminated by interference, solar noise or ionospheric scintillation were removed by a two-stage interactive program2 and the r.m.s. scintillating flux density, ΔS , was then found for the transit of each source.

To detect interplanetary space disturbances it was first necessary to determine the average systematic dependence of ΔS on solar elongation, ε , for each source as accurately as possible. Previous work³ had shown that the variation of ΔS with ε depended on the angular size of the source, rising more

steeply near the Sun for smaller sources, but measurements over 8 yr (during 1967-74) revealed no detectable solar cycle variations, nor has any significant ecliptic latitude dependence been found⁴. 250 strongly-scintillating sources were selected and ~400 daily observations of each were used to plot individual $\Delta S(\varepsilon)$ functions; smooth curves were fitted through the points by means of Chebyshev polynomials, thereby avoid-



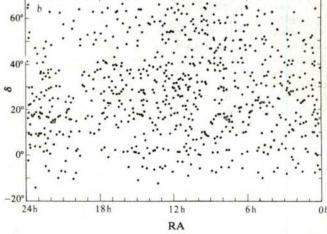


Fig. 1 a, Daily values of the r.m.s. scintillating flux density, ΔS , plotted against solar elongation, ε , for a typical source. b, The grid of radio sources observed.

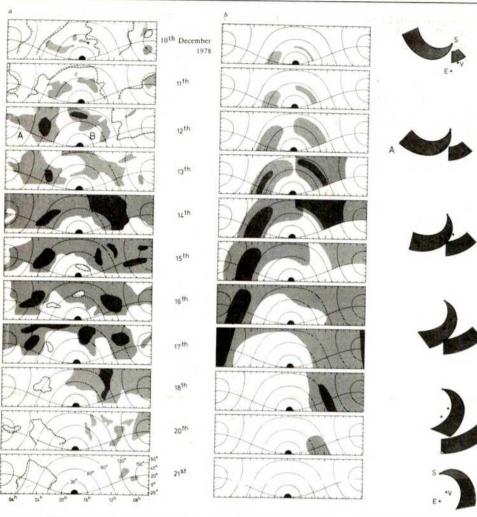


Fig. 2 a, Maps of the disturbance factor, g, across the sky. Light shading 1.25 < g < 1.5; dark shading g > 1.5. The fenced contour lines denote g < 0.5. The Sun is shown as a 15° diameter disk. b, Theoretical maps computed for the model illustrated in c. Schematic diagram of the assumed model. See also Fig. 3b. S, V, E denote the Sun, Venus and Earth.

ing possible errors due to averaging over intervals of finite elongation. The sources were next sorted into groups according to the value of the ratio $\Delta S(\max)/\Delta S(90^\circ)$ derived from the smoothed curves. Having thus separated the sources according to their angular size, daily points for the sources in each group were combined, after scaling to the same mean value of ΔS , to provide smoothed $\overline{\Delta S}(\varepsilon)$ curves. In this way we obtained a set of standard $\overline{\Delta S}(\varepsilon)$ curves against which to compare the day-to-day values of the full grid of more than 900 sources. Our method of normalization avoided the necessity of assuming some particular model of the solar plasma, although the $\overline{\Delta S}(\varepsilon)$ curves were very similar to those derived from such a model A typical $\overline{\Delta S}(\varepsilon)$ curve through the day-to-day points for a source is shown in Fig. 1a.

Interplanetary disturbances were detected by calculating daily values of the disturbance factor, $g = \Delta S/\overline{\Delta S}(\varepsilon)$, for each source in the grid shown in Fig. 1b. Maps illustrating the variation of g across the sky are shown in Fig. 2a, where approximate contours have been fitted by eye to the data. The maps are centred on the position of the Sun so that the scale of right ascension, indicated on the frame for 21 December, shifts from day to day. Note that data for 19 December are not available. The observed declination range is -10° to $+70^{\circ}$, but the frames extend to declination -20° to include the Sun, enlarged to 15° diameter, on the ecliptic. To aid visual interpretation, lines of constant solar elongation at 30° intervals are also shown. Note that the maps are not instantaneous images of the sky. The measurements take 24 h, from west to east, so that the appearance of rapidly-moving features may be distorted, as in a camera with a focal plane shutter.

Features are included on the maps only when we believe them to be real. For example, on 16 December, the small patch of decreased scintillation at $\varepsilon \sim 60^\circ$ due north of the Sun shows a well-correlated variation on all 13 sources within this zone.

When the edges of features take simple shapes, such as the boundary of the enhancement at $\varepsilon \sim 90^\circ$ on the ecliptic west of the Sun on 17 December, they can be located to an accuracy of $\pm 5^\circ$. The extent to which further details have been blurred may be assessed from (1) the relatively coarse contour intervals adopted in this preliminary study and (2) the fact that wiggles in the contours have been drawn only when they embrace correlated variations on at least five sources.

To interpret the maps in terms of three-dimensional structures in space, we first consider the constraints imposed by the theory of propagation through random media. *In situ* measurements on spacecraft^{5,6} show typical plasma density enhancements of two to three times the mean density which last for about 1–3 days. On the assumption, to be justified later, that the small-scale turbulence responsible for scintillation increases in direct proportion to the large-scale plasma density, we now consider the effect of some simple structures consistent with spacecraft data.

Figure 3a shows the location of the important scattering zones along any line of sight. The diagram refers to a particular model³ of the solar plasma but is mainly controlled by the inverse-square dependence of density with solar distance. Spherical symmetry has been assumed, for which there is good evidence over heliocentric latitudes up to 60° (ref. 4). Within a cone defined by $\varepsilon \le 45^{\circ}$ no enhanced scintillation is expected because this is the zone where scintillation saturates due to the onset of strong scattering and blurring of the diffraction pattern by sources of finite diameter. It is this which causes $\Delta S(\varepsilon)$ to decrease for $\varepsilon < 30^{\circ}$, as shown in Fig. 1a. The broken line in Fig. 3a indicates the limit beyond which any disturbance of twice the average density would give g < 1.25 and hence escape detection in our present data reduction.

Consider first a single cloud, about 0.5 AU in diameter when it reaches 1 AU, in which the density is twice the mean density

at any solar distance. Travelling at typical solar-wind speeds the cloud would cover a distance of 1 AU in about 4 days so that on successive days it might appear as shown in Fig. 3a. For lines of travel at angles $\phi \ge 90^{\circ}$ to the Sun-Earth direction, the cloud would be undetectable, except perhaps for about 1 day along lines of sight such that $\varepsilon \sim 45-60^{\circ}$. For $\phi < 90^{\circ}$ the cloud would be detectable for about 5 days. The largest enhancements of scintillation would be seen at $\varepsilon \ge 90^{\circ}$ when the cloud best fills the main scattering zone, which occurs for ϕ < 45°. To produce a disturbance lasting longer than about 5 days, the Sun must eject a succession of clouds or a continuous stream. This would take the form of a co-rotating sector, due to solar rotation, as sketched in Fig. 3a. A long-lived enhanced density disturbance of this type would be seen first to the east of the Sun and then to the west, and would be detectable for 10-12 days in all.

We found that the above constraints enabled the general form of the three-dimensional structure of the observed enhanced scintillation zones to be determined with little ambiguity. Figure 2a shows a persistent enhancement to the east (labelled A) at $\varepsilon \sim 90-150^\circ$ which lasted for 8 days. This can be explained only by the approach of a co-rotating stream. The sector must cover about $30-45^\circ$ in heliocentric longitude to account for the wide range of elongation over which it was seen on 12-13 December, and its position in longitude can be determined to $\sim \pm 5^\circ$ from the location of the sharp trailing edge, seen at $\varepsilon \sim 60-90^\circ$ near the ecliptic during 15-17 December. The extent of the sector in heliocentric latitude, which can be gauged from the rapidly increasing northward ecliptic latitude observed on 10-12 December, must be at least 45° .

The co-rotating disturbance cannot account for the enhancement (labelled B) seen above the Sun on 10-11 December and

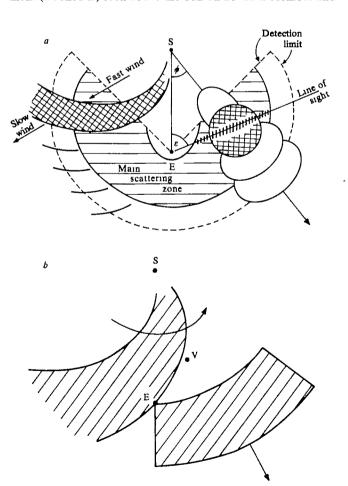


Fig. 3 a, Schematic diagram showing the location of the dominant scattering zones along any line of sight. b, The configuration of the model disturbances on 14 December.

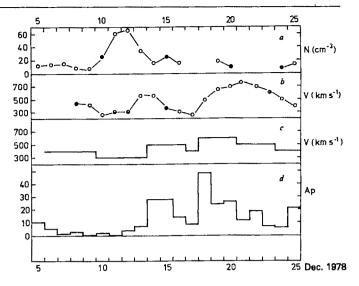


Fig. 4 a, Daily values of the solar plasma density observed near Venus. b, Daily values of the solar wind speed near Venus at about noon UT. c, Daily average solar wind speed near Earth.

d, Daily average magnetic indices, Ap.

expanding over a wide range of ecliptic latitude on 12-13 December. A and B remain clearly separated on the maps until 14 December, and the co-rotating feature discussed above could not possibly give rise to enhanced scintillation on the ecliptic at $\varepsilon \sim 90^{\circ}$ to the west on 13 December. Disturbance B. was extended roughly along lines of constant elongation and was most prominent at $\varepsilon \sim 75-120^{\circ}$ to the west on 14 December over ecliptic latitudes -20° to +90°. These characteristics indicate a tangentially extended disturbance passing Earth to the west and cutting off near the Sun-Earth line. The radial thickness of the shell must have been ~0.5 AU at a distance of 1 AU to explain the range of elongation of the enhancement on 12-13 December. Its radial velocity, derived from the outward motion of the leading edge, was $350 \pm 50 \text{ km s}^{-1}$, and the shell must have been about to engulf the Earth on 12 December. The notable increase of the enhancement on 14 December, which we interpret as due to the tangential alignment of the line of sight with the shell, indicates a spread in heliocentric longitude of at least 45°, and a corresponding extent in heliocentric latitude above the ecliptic.

To confirm these semi-quantitative estimates, we adopted the simple model shown in Fig. 3b and assumed that within each disturbance the density was doubled. The shell covered 50° in longitude and latitude above the ecliptic, and travelled at 350 km s⁻¹. The spiral envelope of the co-rotating sector corresponded to a velocity of 430 km s⁻¹ and the sector covered latitudes ±50° and 45° in longitude. In this simple analysis we ignored any interaction between the disturbances, such as might have occurred after 14 December, and computed the scintillation adopting weighting functions along the line of sight mentioned earlier. The contributions of each disturbance may be distinguished in the theoretical maps shown in Fig. 2b. Note that the shell has passed from view after 15 December. Our model simulates the observations remarkably well, considering its simplicity, and we do not believe that it could be significantly modified. Details such as the sphericity of B are not accurately known.

Before comparing our conclusions with other space and geophysical data we return to our assumption that enhanced scintillation correlates with enhanced plasma density. Much evidence now exists that enhancements of scintillation may be identified with regions of compressed plasma moving outwards from the Sun in advance of high-velocity zones. The compression regions usually occur between fast and slow solar wind streams^{7,8} or, less often, near the leading edges of disturbances associated with solar flares^{9,10}. In some cases there is evidence

that scintillation enhancements may be displaced slightly from the zone of maximum density11.

Relating our present observations to previous work, we infer that the high-velocity stream generating co-rotating disturbance A must arrive at the Earth around 17-18 December, following the departure of the compression region. The observation of a zone of decreased scintillation to the east during 18-21 December supports this conclusion, as the plasma density within fast solar wind streams has less than average density⁵. Disturbance B, which clears the Earth around 14 December, should also be followed by a zone of increased velocity.

Daily average values of spacecraft measurements and associated geomagnetic data¹² are presented in Fig. 4, together with solar plasma measurements near Venus by the Pioneer-Venus Orbiter¹³. All these observations are in excellent agreement with our model, providing further confirmation that enhanced scintillation relates to increased plasma density. A fast solar wind stream arrived at the Earth on 18 December and initiated strong geomagnetic activity. This stream was associated with a coronal hole of positive polarity which appeared on the Sun about five rotations earlier¹². Another high-velocity zone of shorter duration reached the Earth on 14 December and again caused a geomagnetic disturbance. Finally, we note that our model predicts the arrival of enhanced density at Venus on 10 December, and its departure on 12-13 December. The observed density follows this pattern, and the high-velocity zone reached Venus on 13 December, about 1 day before reaching the Earth, A shock wave at Venus was observed at 07.50 UT on 13 December, and at 01.27 UT on 14 December at the Earth¹³. This shock has been tentatively associated^{13,14} with solar flares commencing either at 23.32 UT on 10 December in McMath plage region 15697 or at 18.32 UT on 11 December in region 15694. As the wind speed rose to 500 km s⁻¹ within a few hours after the arrival of the shock at Venus, and as the shock corresponded closely with the inner boundary of disturbance B, we assume that these are related events. It is evident, however, that disturbance B could not have been generated by either of the flares because its leading edge had already advanced beyond 0.5 AU from the Sun by 12.00 UT on 10 December.

Our results demonstrate the potential of scintillation as a method of studying the morphology of interplanetary disturbances in three dimensions. The ability to track disturbances in the range 0.5-1.5 AU should be useful in the field of solarterrestrial relationships, and as a technique for predicting geomagnetic storms. The forecasting of magnetic activity depends, of course, on the early recognition of disturbances that will later engulf the Earth. In the present case we believe that the salient features of disturbance B, including the likelihood that it would hit the Earth, were evident by about 12 December, giving 2 days warning of the magnetic storm on 14 December. Similarly for disturbance A, the co-rotating nature became clear by 13 December, giving 5 days warning of the storm on 18 December.

G.R.G. and A.P. thank SERC for the award of a studentship and postdoctoral fellowship respectively.

Received 18 November 1981; accepted 24 February 1982

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Wave-produced bubbles observed by side-scan sonar

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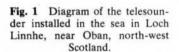
When a wave breaks in deep water, forming a whitecap, many small bubbles are generated and carried below the surface by turbulence. The breaking wave forms a strong acoustic target when viewed from below by an inverted side-scan sonar. The bubbles remaining in the water after the breaking wave has passed out of the sonar beam are also good acoustic scatterers and their drift towards or away from the sonar provides a means of remotely measuring that component of the near-surface current without interference of the flow and without exposing instruments to the hazard of violent near-surface conditions. This current changes rapidly as the wind changes. The sonar display shows regions of convergence or divergence of bubble clouds, perhaps associated with Langmuir circulations, and may, in calm weather, respond to the presence of surface slicks.

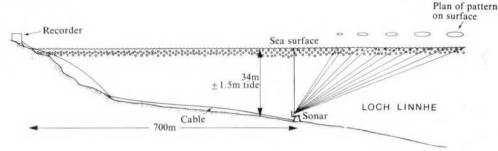
We have used an inverted telesounder¹, a modified side-scan sonar, to probe the sea surface and near-surface scatterers. The sonar consists of a single linear 248 kHz transducer mounted horizontally a few centimetres above a reflecting plate inclined upwards from the horizontal at about 20° (Fig. 1). Pulses of sound 0.08 ms (12 cm) long are transmitted at 0.25 s intervals in a vertical plane in a narrow beam which is modulated by interference with sound reflected from the plate. The pattern of the effective beam at the surface consists of a series of ovals extending away from the sonar, typically about 1 m wide and with length increasing from 1 to \sim 5 m.

The sonar was mounted by divers on a frame resting on the sea bed in Loch Linnhe, north-west Scotland, at a depth of 34 m and some 700 m from shore, to which it was connected by cable (see ref. 2 for details of earlier experiments and site). Recording was on a conventional graphic recorder displaying time versus the time delay between transmission of sound and reflected signal or effectively, for uniform sound speed, time versus range of scatterers. The beam was directed towards 290° T (true north) and, during the period of observation, the wind and swell were approximately from 225° T.

Figure 2a shows the display in conditions of low wind speed (figure times are given in Fig. 3). The return from the sea surface directly above the sonar is shown by the distinct lower dark band; in the original record both swell and wind waves can be distinguished, with periods of about 8 and 2.5 s respectively. Above the surface (at greater range from the transducer) appear a series of bands corresponding to sound scattered from the ovals on the sea surface. Their mean position is in reasonable accordance with the prediction of sound intensity shown on the right of Fig. 2a (see ref. 1 for theory). Undulations can be seen which correlate with the swell in the direct surface return. The fading of the record at the beginning and the end may be due to slicks which reduce the surface capillary waves and hence the effective scattering at 6 mm sound wavelength.

In windy conditions, the bands are overlain by parallel streaks which trend towards, or away from (as in Fig. 2b), the sonar and last for 1-5 min. Figure 2c covers a period of rapid change in pattern during a squall whilst Fig. 2d shows a more gradual change in streak direction. The streaks often start with a sharp dark line, indicating a strong scatterer moving at about 1.7 m s in these examples towards the sonar. Occasionally two such





lines are present (B, Fig. 2d). The streaks occasionally continue between bands (see, for example, Fig. 2b) indicating that the scatterers are not all in the surface ovals (as in Fig. 2a) but below the surface at shorter ranges.

The speed with which the scatterers producing the streaks move parallel to the sea surface towards the sonar is plotted in Fig. 3, together with wind speed and direction, and tidal variation (measured by the sonar). The streak speed is much less than that of phase speed of the waves (about 12.5 m s⁻¹ for swell and 3.9 m s⁻¹ for wind waves) although the lines commencing the streaks have speeds approximately equal to the component of the wind wave phase speeds in the direction of the sonar beam, which, like the wind, were always directed towards the sonar in the examples shown here. We deduce that the lines are the breaking crests of waves that produce trailing clouds of bubbles which, left in the water, are then advected by the near-surface current to form the streaks.

These bubbles have been described elsewhere^{3,4}. Most bubbles have radii of $20-150 \mu m$, have rise speeds of $<1 \text{ cm s}^{-1}$ and are concentrated near the surface². Their scattering cross-section decreases exponentially below the surface in vertical distances of 0.4-1.0 m in winds of $4-12 \text{ m s}^{-1}$. At a horizontal

range of 60 m their rise speed would contribute < 0.5 cm s⁻¹ to the current away from the transducer, which is within the accuracy to which the bubble drift can be determined from the sonar display (~1 cm s⁻¹). The speeds shown in Fig. 3 may thus be identified, to within the experimental error, as the component of the near-surface current towards the transducer. The current varies rapidly with change in wind speed (Fig. 2c). At wind speeds of about 7 m s⁻¹ (Fig. 2b) the waves break so frequently that most of the near-surface water contains bubbles which produce detectable scattering. No obvious regular patterns (like, for example, cloud streets) can be seen and the bubble population at a particular location appears to be determined principally by the earlier presence of breaking waves and advection. Clouds are typically 1-2 m wide and little lateral diffusion is apparent. Indeed, most of the streaks become gradually narrower with time, which suggests that the reduction in acoustic scattering cross-section of the clouds, due to bubbles returning to the surface or dissolving, occurs more rapidly than their lateral spread by turbulent diffusion. Small-scale convergences and divergences of the bubble streaks are, however, visible (for example at A, Fig. 2b), and these may be associated with Langmuir circulation patterns5.

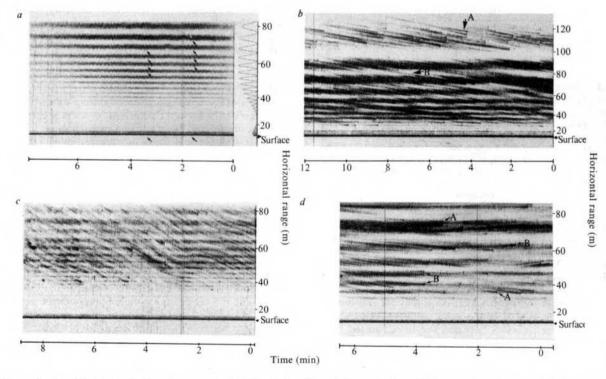


Fig. 2 Sonar displays. Note that the time increases to the left and that the non-linear horizontal distance along the surface is shown at the right. a, Low wind speeds. The arrows indicate groups of swell. The calculated intensity of a return from a uniform field of reflectors at the surface is shown at the right. The band of marks 2 min after the start of the record is due to an unknown noise source. b, The scatterers, identified as clouds of bubbles producing the streaks in the bands, are moving away from the transducer. Converging scatterers are shown at A whilst the scatterers at B are between the surface scattering bands and are therefore sub-surface. c, A squall showing a rapid variation in streak pattern. Compare with a and note the presence of (sub-surface) scatterers (bubbles) between the surface bands, especially at 4 min and 50 m range. d, A shift in current producing a change from approaching to receding streak pattern. Note the single line (breaking wave crest) commencing the streaks at A and double lines at B, where two waves in succession have broken within the sonar beam,

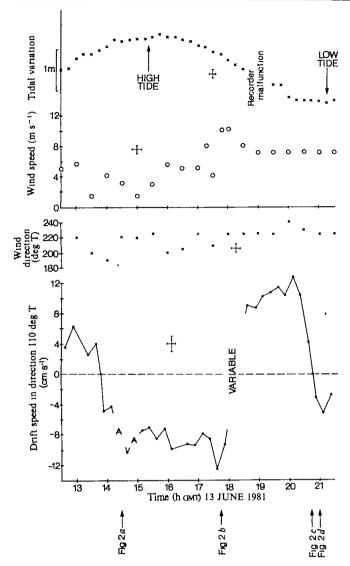


Fig. 3 The variation of tidal variation (measured from the sonar relative to an arbitrary zero), wind speed and direction (recorded at the laboratory of the Scottish Marine Biological Association, Dunstaffnage, ~1.1 km south-east of the sonar) and the 15-min average drift speed of scatterers in direction 110° T. The times corresponding to Figs 2a-d are noted on the time axis. At some times (marked A; for example, the period shown in Fig. 2a) no streak lines can be seen because the wind is not strong enough to produce breaking waves and bubbles which can be detected by the sonar.

Much of Fig. 3 can be explained in terms of wind and tide. The positive component up to about 1330 h is consistent with both wind and tidal flow. A southwesterly tidal flow should develop 1 h 50 min before high tide⁶ and indeed appears as a negative component at 1345 h slightly delayed, perhaps by the opposing wind. The increasing wind at 1745 h produces a change in current direction which continues for 3 h but then

The mean time intervals between the lines formed by the breaking crests decrease with increasing wind speed and are in accordance with earlier observations of bubble clouds⁷. Wind waves are, however, seen to break more frequently than bubble clouds appear to be generated, and it appears that only the more vigorously breaking waves may be detected by the present sonar.

The method of determining near-surface currents by remote acoustic sensing may have particular value in severe wave conditions when the signal-to-noise ratio is enhanced because more bubbles are produced and when conventional moored instruments are prone to considerable hazard and error due to mooring motion. The technique may be useful in measuring the sea state and frequency of wave breaking, as well as perhaps monitoring slicks or coastal fronts and internal waves which modulate the near-surface currents.

Received 13 October 1981; accepted 19 February 1982

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Storm trajectories in eastern US D/H isotopic composition of precipitation

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The isotopic content of precipitation falling at a point provides information of potential importance concerning atmospheric circulation and climate change 1-6. The isotopic content of precipitation, however, is controlled by the particular meteorological processes acting at a given time and place and is subject to considerable variation. We have measured the deuterium-tohydrogen ratios (D/H) of precipitation at Mohonk Lake, New York, from individual storms over a 2-yr period, July 1977-June 1979. These ratios varied systematically with the locus of the paths followed by the storms: the more seawards (and southerly) the paths and the colder the temperatures at Mohonk, the lower were the D/H ratios.

Very large differences in the D/H ratios from storm to storm were observed. The δD range for all storms was -7% to -170% $\delta D = (D/H \text{ sample} - D/H \text{ SMOW})/(D/H \text{ SMOW}) \times 10^3$ where SMOW is standard mean ocean water. In general the storms with the highest deuterium contents occurred during the warm summer and early autumn months while the storms with the lowest deuterium contents occurred during the cold winter

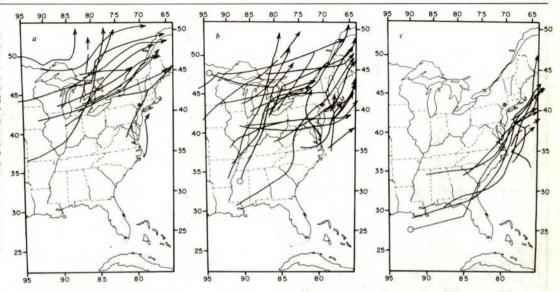
There was a large to small range of δD values during single storms. During two storms on 20-21 January 1978 and 25-26 January 1978, precipitation was also sampled sequentially at a separate location in Palisades, New York. For the storm of 20–21 January, the δD for the first 2 inches of snow was -177%, while the ice crust formed by a mixture of the later snow with freezing rain that fell at the end of the storm had a δD of -75%. For the storm of 25-26 January 1978, the δD for the first $\frac{1}{2}$ inch of rain was -78% compared with -54% for the last $\frac{1}{2}$ inch. We detail elsewhere how meteorological analysis can be used to explain the δD ratio of cyclonic precipitation at a given time.

We have found that a strong relationship exists between the isotopic content of precipitation at Mohonk for a given storm and the path taken by the storm (the storm track or trajectory): in general the 8D decreases systematically as the locus of the storm trajectories is displaced seawards (Fig. 1).

Storms in the eastern US are frequently produced as a result of the interaction of cool, dry continental air with warm, humid maritime air. There is often a rather narrow and sharp transition zone, known as the polar front, that separates these two distinct air masses. Cyclones occur more frequently along the polar front.

During the colder half of the year when the area of polar influence expands and the meridional temperature gradient is

Fig. 1 The trajectories of the low pressure centres of storms occurring between July 1977 and June 1979. The trajectories were separated into five groups on the basis of the 8D of the precipitation that fell at Mohonk Lake near New Paltz, New York. Three of the groups are shown: a, δD≥ -30%; b, $\delta D = -61$ to -90%; c. $\delta D \leq -121\%$. The two intermediate groups (isotopic ranges: -31% to -60%, and -91% to -120%) not shown have trajectories intermediate to those shown. S, The location of Mohonk Lake. O. The origins of two storms of January 1978 from which a sequence of samples was taken at Palisades, New York.



larger, the polar front is typically located further south and is more often well defined. Accordingly during the winter both cyclone frequency and intensity are greater and the average position of the paths taken by the cyclones are located further south. For the eastern US this means a greater percentage of storm tracks with coastal or offshore positions during the winter.

Similarly, during the height of the ice ages, the area of polar influence was greatly expanded and the average position of the storm tracks was much further south than today⁸. Therefore a greater percentage of the ice age storms would have been classified as coastal storms and would have produced precipitation at New York and on the North American ice cap with correspondingly low values of δD .

There is a straightforward explanation for this relationship between the δD of precipitation and position of the storm tracks. Air undergoing condensation from cooling due to lifting experiences a progressive depletion of the heavy isotopes ^{1,9,10}. Thus the precipitation from a given air parcel that forms last, or at greatest altitudes, invariably has lower δD and $\delta^{18}O$ values than any earlier precipitation from that air parcel ¹. When a frontal surface is aloft, most of the precipitation forms in the air above the front. It follows then that the higher the frontal surface, the lower the δD of precipitation tends to be. In general the higher the frontal surface above a particular location, the further south the storm is from that point. Storms that stay far to the south or remain well seawards of New York will tend to have the lowest δD because the frontal surface is always found at considerable altitude.

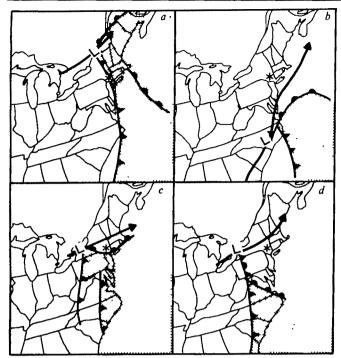
These observations lead us to predict a coherent spatial distribution of δD values around a typical extratropical cyclone. The D/H ratios should be highest for the precipitation in the warm sector of the storm, although they may be subject to some variations because much of the precipitation in the warm sector can be convective. The steep slope of the cold front should cause a dramatic decrease of the δD to the west of the surface position of the cold front (such behaviour has been described elsewhere 11). Northwards of the warm front there should be a coherent but gradual decrease of δD consistent with the gentler slope of the warm front.

Such a synoptic pattern of δD values can be used to account for the rather wide range of storm track positions for those storms producing intermediate δD values (as in Fig. 1b). Two storms may have very similar tracks but quite different δD values if the positions of the warm fronts are different. This is illustrated in Fig. 2, in which four separate storms together with their storm tracks are shown. When storm tracks are either far north or far south of Mohonk, there is little ambiguity about the δD values. However, for storms with intermediate tracks, the position of the warm front is critical for determining δD .

Figure 2a shows the storm of 9 January 1978; the track lay far north of Mohonk Lake and, as is characteristic for such

Table 1 8D of precipitation from storms

July 1977	July 1977 to June 1978			July 1978 to June 1979			
Date	Amount (inches)	8D (%)	Date	Amount (inches)	8D (%)		
6 July	0.27	-35 ± 2		1.12	-84±5		
		-33 ± 2 -21 ± 2	3-4 July		7		
8 July	0.17		10 July	0.33	-40±2		
17 July	0.17	-12 ± 1	17-18 July	0.33	-38±1		
19 July	0.20	-25 ± 1	24 July	0.24	-28±1		
25 July	0.70	-33 ± 1	28-29 July	0.29	-20±		
1-2 August	0.12	-22	31 July-1 August	0.38	-56±3		
3 August	0.17	-44	4 August	0.20	-45±2		
5 August	0.18	-35	6-7 August	1.33	-38 ± 2		
12 August	0.27	-14	11-12 August	0.34	-44±2		
17 August	0.41	-26	24-25 August	0.75	-7±		
22 August	0.17	-39	28 August	0.18	-17 ± 2		
31 August	0.16	-29	31 August	1.62	-45 ± 1		
13-14 September	0.53	-18	12 September	0.31	-24±2		
16-17 September	1.27	-46	18-19 September	2.31	-35 ± 1		
20-21 September	1.78	-50	22-23 September	0.25	-12		
23-26 September	4.10	-65	4 October	0.22	-25		
1-2 October	2.26	-43	6-7 October	0.70	-29		
8-9 October	1.32	-11	25-27 October	0.40	-22		
14-15 October	0.59	-21	7-8 November	0.16	-37		
16-17 October	1.00	-14	17-18 November	1.12	-31		
19-20 October	0.51	-47	23-24 November	0.68	-67		
7-9 November	5.18	-44	29-30 November	0.30	-161		
25-26 November	1.22	-88	3-4 December	0.42	-83		
29 November-			8-10 December	1.42	-84		
1 December	1.02	-62	21-22 December	0.95	-92		
9 December	0.21	-66	24-25 December	1.51	-112		
12-15 December	1.49	-40	1-4 January	2.79	-62		
18-19 December	0.51	-146	5-6 January	0.18	-170		
20-21 December	0.89	-58	7-8 January	2.30	-89		
25 December	0.32	-73	12-14 January	0.50	-81		
1-2 January	0.31	-113	17-18 January	0.25	-99		
6-9 January	2.50	-48	20-21 January	2.91	-87		
13-14 January	1.20	-116	24-26 January	2.18	-86		
17-18 January	1.38	-96	7-8 February	0.32	-127		
19-20 January	0.89	-145	19 February	0.32	-170		
25–26 January	1.56	-75 ± 1	23-25 February	1.70	-69		
6-7 February	0.76	-138	25-23 February	1.00	-109		
3 March	0.70	-123	2 March	0.18	-72		
14 March	1.31	-123	6-7 March	1.33	-45		
16 March	0.16	-130	10-11 March	0.60	-110		
25–27 March		-55	14 March				
	2.38			0.10	-39		
19-20 April	1.25	-55 ± 2	24-25 March	0.82	-46		
5-6 May	0.80	-134 ± 2	29 March	0.22	-55		
9 May	0.80	-50 ± 2	2-3 April	0.27	-30		
10 May	0.75	-34 ± 1	4-5 April	0.24	-66		
14-15 May	1.97	-31 ± 2	8-10 April	1.27	-64		
16-18 May	1.91	-67 ± 2	14-15 April	1.10	-63		
24-25 May	2.34	-57 ± 2	26-28 April	1.38	-37		
31 May	0.40	-22 ± 1	3-4 May	0.22	-29		
3 June	0.72	-51 ± 4	12-14 May	0.59	-42		
7-8 June	0.92	-21 ± 4	18-20 May	0.26	-50		
9 June	0.45	-62 ± 2	23-25 May	4.30	-53		
13 June	0.16	-38 ± 2	26 May	0.26	-48		
21 June	0.72	-21 ± 4	5 June	1.01	-46		
			11 June	1.74	-41		
			18 June	0.36	-21		
			30 June	0.21	-20		



the 8D values are given for four different storms. a, 9 January 1978, 12.00 GMT, 8D = -48; b, 20 January 1978, 1.00 GMT, 8D = -145; c, 15 December 1977, 00.00 GMT, 8D = -44; d, 2 January 1978, 00.00 GMT, 8D = -145; d, 20 January 1978, 00.00 GMT, 8D = -113 The shaded region shows the warm sector of the storms. Heavy arrows show storm trajectories. *, The collection site at Mohonk Lake, New York.

storms, most of the precipitation at Mohonk occurred as rain in the warm sector or near the warm front. Such storms mostly produce a high δD.

This is to be contrasted with the storm of 20 January 1978 (Fig. 2b), which took a far more southerly track. Throughout this coastal storm, Mohonk Lake remained deeply embedded in the cold air. Characteristically, the precipitation fell amost entirely as snow (it was a major snow storm for the entire northeastern US) and 8D was very low.

Figure 2c, d shows two storms with intermediate and almost identical tracks but with a large difference in the 8D values for each storm which is a direct result of the difference between the positions of the warm fronts for each storm. The storm of 15 December 1977 (Fig. 2c) had a quite high value of δD. Most of the precipitation fell when the warm front lay only a short distance south of Mohonk. In contrast, the warm front for the storm of 2 January 1978 (Fig. 2d) remained well south of Mohonk through the time of the precipitation and consequently the 8D value for this storm was much lower.

We have also found a significant relationship between the δD values and the mean effective temperature during the precipitation. The quantity \bar{T} is calculated by weighted averaging of the temperature using precipitation amounts. Then, for January, storms with at least 0.75 inches of precipitation, we obtain

$$\hat{\delta}D = -119.5 + 5.0\bar{T} \tag{1}$$

where δD is the expected value of δD (in %) and \bar{T} is in °C. The correlation coefficient is 0.91, which is surprisingly high considering that we are relating a surface parameter to a process occurring above ground level. For most large January storms the principal maritime source is the warm waters extending from the Gulf of Mexico through the Caribbean Sea to the Atlantic Ocean. Source conditions vary rather little from storm to storm but temperatures at New York may vary considerably. Apparently the temperature at New York during times of precipitation in January varies coherently with the frontal position and with the important precipitation producing processes aloft.

Two distinct results of the present findings have great potential value. First, the isotopic composition of the precipitation from a given storm depends strongly on the details of the storm's path, structure and evolution. We have shown previously that by taking these details into account, we can calculate accurately the isotopic composition of precipitation falling at a given time and place. This shows that the δD values of precipitation or vapour in the air act as a tracer of atmospheric motions. The δD value depends most strongly on the vertical profile of the ascent rate of the air in the cloud which cannot be measured directly at present. The measurement of δD at a given time. therefore, provides a means for estimating the vertical profile of the ascent rate of the air in the clouds above.

Second, a climatic signal can be extracted from the δD values of precipitation from storms. Changes in atmospheric circulation redistribute the trajectories of storms (Fig. 1) and/or the amounts of precipitation (Table 1) in both time and space. An integrated sample of precipitation or a portion thereof provides the isotopic signal recorded in tree rings, freshwater fossils or glacial ice cores. Routine analysis of all storms would provide a better foundation for evaluating climate change from isotope

We thank W. D. Donn, E. R. Cook, G. Jacoby and W. S. Broecker for valuable discussions. This research was supported by grants ATM-77-19217 and ATM-79-18920 from the NSF.

Received 30 November 1981, accepted 8 February 1982

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A 1.2-Myr record of isotopic changes in a late Pliocene Rift Lake, Ethiopia

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The Hadar fluvio-lacustrine formation (Ethiopia) contains an original hominid fossil Australopithecus afarensis and an abundant vertebrate faunal assemblage^{1,2}. The formation^{3,4} consists of a 150-300 m thick sequence of fine-to-coarse detrital sediments, originating from the Ethiopian Plateau escarpment, with some interbedded pyroclastic layers. The three lower members of the formation were formed in a lacustrine environment, which was occasionally interrupted by fluvial drainage. The upper member is quite distinct and formed as an alluvial fan delta. This sedimentary sequence was deposited at the end of the Pliocene between 3.9 and 2.7 Myr, as indicated by magnetostratigraphy⁵ (the palaeomagnetic record spans the Gilbert-Gauss transition period), K/Ar dating^{6,7} and biostratigraphy⁸. We consider here the oxygen and carbon isotope variations observed throughout the formation and their palaeohydrological implications.

Eighty-six carbonate horizons and 28 mollusc-bearing units were collected throughout the section, from the base to the top. The carbonates are mainly lacustrine nodules. However, at the base of the first member some micritic limestone beds occur and one horizon of the second member contains stromatolitic structures (oncolithes). These carbonates consist of a low magnesian calcite.

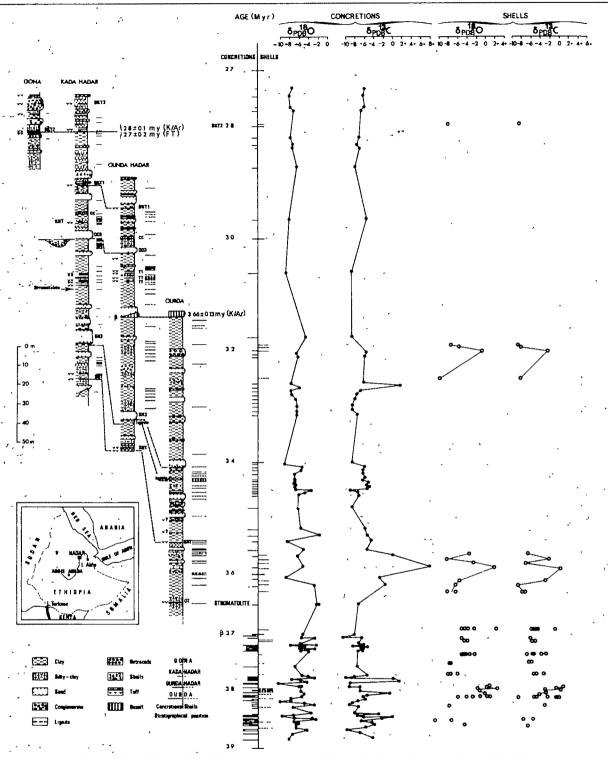


Fig. 1 The Hadar Formation. Lithology, chronology and isotopic changes in concretions and fossils.

The fossils which were collected are essentially shallow freshwater gastropods (*Melania tuberculata*, *Bellamya* sp., *Cleopatra* sp.) and a bivalve (Unionidae). The shells are usually pure aragonite and do not show any evidence of post-depositional recrystallization.

The δ^{18} O_{PDB} values, from the base to the top of the section (Fig. 1), show large fluctuations between -10.8 and -2% for concretions and between -10.5 and 2.5% for shells. These values indicate highly variable palaeohydrological conditions during the episode, with phases of drastic evaporation inducing considerable enrichment of the water in heavy oxygen. The shells are systematically enriched in 18 O compared with the concretions. This enrichment reflects both the aragonitic nature of the shells which represents a 0.8% difference with calcite

and the adaptation of molluscs to highly evaporated waters. For example, five species found within one single layer of the second member (Fig. 1) have δ^{18} O values ranging from -6 to 1%. Specific fractionation cannot explain such large differences, which are more realistically explained by environmental changes both in time and space, with conditions ranging from freshwater to highly evaporated and eventually brackish water. Most of these molluscs currently tolerate such conditions (see, for example, refs 10, 11 for Pleistocene Saharan lakes, or ref. 12 for rift lakes). That hydrological changes were very frequent in the Hadar lake, is shown by the 18 O variations in the stromatolitic structure (Fig. 2). It is difficult to assess any periodicity for the growth of oncolithes, but seasonal or annual cyclicity might be considered for those of biogenic origin 13,14 ,

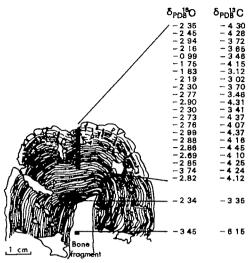


Fig. 2 Isotopic variations in one of the oncolithes of the second member.

which seems to be the case here as indicated by the ¹³C content (see below). Thus within a few years, changes as high as 3% (Fig. 2) occurred in the ¹⁸O content of calcite, these should essentially reflect variations of the isotopic composition of the lake water. An increase in temperature is not likely to be responsible for a large negative shift of ¹⁸O content in carbonates, because it would be largely counterbalanced by a positive effect associated with increased evaporation. Negative shifts of ¹⁸O values are more realistically explained by influx from precipitations. Consequently, positive shifts, from the lowest δ^{18} O values to higher ones, must essentially be the result of evaporation periods which followed the precipitations. Thus variations in the 18O content of carbonates can be related to contrasted hydrological regimes. Abundant precipitation induced sudden lake level rises; the isotopic composition of the water during such episodes was occasionally as low as $-12\pm1\%$ (SMOW), if we accept for calcite precipitation an average temperature ~25±5°C, as suggested by faunal requirements15. Periods of high lake levels were rapidly followed by isotopic evolution typical of closed basins: the evaporation induced an enrichment of the water in ¹⁸O, with δ ¹⁸O values as high as 0%. Such rapid hydrological changes suggest that the lake was mainly fed by strong floods coming from the Ethiopian Plateau escarpment. This agrees with the facies distribution which also indicates rapid changes in the sedimentary dynamics.

Similar hydrological regimes were observed in nearby Holocene lakes by Fontes and Pouchan¹⁶ or Gasse *et al.*¹⁵. The δ^{18} O values of Lake Abhé water for the Holocene¹⁷ are close to our mean values, although the range of variation there (-11.9)to -5.8%) is not as large as it was in the Pliocene Hadar lake. The similar morphological situation in the Afar Rift, and the strong influence of runoff and drainage from the Ethiopian Plateau are common to both lakes. However, the higher δ^{18} O values which were occasionally present in the Hadar lake water suggest stronger evaporation phases at the end of the Pliocene leading occasionally to subaerial conditions. The occurrence of the flood plain facies which sporadically interrupt the lacustrine sequence of the three lower members supports this interpretation. The upper member, which is characterized by coarse alluvial fan deposits, does not show large isotopic changes. The relatively stable δ^{18} O values of carbonates (-8 to -6%) indicates a fast drainage system without any strong influence of evaporation. Thus the ¹⁸O content of carbonates could reflect the average isotopic composition of the precipitation during this episode ($\delta^{18}O \approx -8.5\%$).

The ¹³C content of carbonates (Fig. 1) suggests a noticeable contribution of biogenic CO₂. A few concretions or shells show positive isotopic compositions for carbon, however, it is difficult to accept equilibrium with atmospheric carbon dioxide. Such positive $\delta^{13}\hat{C}_{PDB}$ values are linked to sedimentological features

indicating a reducing environment (with lignite layers, for example), and thus to an unusual CO₂ metabolism¹⁸

In conclusion, isotopic compositions of the Hadar lake carbonates do not reflect climatic conditions drastically different from those which prevailed during the Holocene in the area15. The lowest δ^{18} O values calculated for lake water indicate either a cold origin of the waters precipitated between 2,000 and 3,000 m on the Ethiopian Plateau, or a pronounced continental effect. A similar meteorological pattern of monsoon rains produced by cooling of humid air masses which could originate from the Gulf of Guinea, the Indian Ocean or exceptionally the Gulf of Aden, may have prevailed during the late Pliocene. The variability observed in the Hadar lake regime strongly suggests either a more contrasted seasonality as it has been proposed for the same epoch in Laetoli, Tanzania¹⁹, or longer term climatic oscillations, or both. The hydroclimatic evolution of the Afar Rift through time seems to differ from that proposed by Cerling et al.12 for the Gregory Rift more to the south in Kenya. Their data in Lake Turkana support the hypothesis of a more humid period during late Pliocene. The inland situation of Lake Turkana could explain the difference between the two areas. However, the limited number of data presented and the various origins of the carbonates (shells, lacustrine and soil nodules) analysed in both areas preclude a detailed comparison of the two sites.

The palaeomagnetic data of the Hadar Formation have been computed again and calibrated with new radiometric dates⁶. The Cochiti events of the Gilbert period and the Kaena and Mammoth events of the Gauss normal period seem to be present in the Hadar sequence. T. J. Schmitt, A. E. N. Nairn, J. L. Aronson, R. C. Walter, M. Taieb, and J. J. Tiercelin are all involved in this new study.

We thank the Ethiopian Government. This study was supported by CNRS, Fr. (A.T.P. 042) and CRSNG, Canada. Comments by H. Faure, J. C. Fontes and J. Bourne considerably improved the manuscript. T. E. Cerling helped to clarify several aspects of this final version.

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Bacteria accumulate silver during leaching of sulphide ore minerals

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Silver is extremely toxic to a wide range of bacteria 1-4, and has even been used in solution to control bacterial growth2. In an investigation of the oxidation of several sulphide minerals containing traces of silver by a mixed culture of Thiobacillus ferroxidans and Thiobacillus thiooxidans, it was noted that the bacteria accumulated silver. I report here that careful collection of most of the cells from such experiments yields a silver

concentrate, while the silver attached to the cells remaining in contact with leach residues is readily recovered by a conventional cyanidation technique. These results suggest that the bacterial leaching of valuable and non-valuable sulphide minerals in order to release atomically bound silver, and possibly gold, may be used as part of a recovery process for these elements.

Electron microscopic examination of bacteria collected from several experimental sulphide leaching systems, in which substrates contained silver, has shown that the bacteria involved in the leaching can accumulate silver in very large quantities and that their activity does not appear to be inhibited by silver contained in sulphide minerals. The manner in which silver is accumulated by the bacteria is illustrated in Fig. 1, which shows an electron micrograph of a single bacterial envelope prepared by washing a mixed culture of active bacteria collected from a sulphide leaching experiment with dilute KOH. The membrane is covered with small electron-dense particles of silver in the form of silver sulphide. These small AgS granules have been observed to grow on the surface of bacteria collected at different stages of a batch leach finally accumulating to form large clumps. Cleaned suspensions of bacteria coated with such particles are very dark compared with normal bacterial suspensions and on drying at 100 °C, a shiny black deposit is produced. The amount of silver accumulated per gram of dry weight biomass varies in proportion to the silver content of the sulphide mineral being leached, but concentrations greater than 250 mg per g have been observed. The granules on the bacterial surface were analysed using a Philips EM 400 electron microscope microprobe analyser and were found to contain silver and sulphur in proportions suggesting the formulae Ag₂S. The granules are crystalline and results of polycrystalline electron diffraction indicate that the compound is the silver mineral acanthite $(Ag_2S).$

When initially observed on the surface of bacteria recovered from a sulphide leaching test, these small silver sulphide particles were considered to have been released from the matrix of the sulphide mineral being leached, and to have remained unoxidized. This hypothesis was supported by the fact that silver sulphide is unaffected by the action of acidophilic bacteria3. Further experimentation, however, has shown that these silver sulphide particles can be produced artificially on the surface of bacteria by adding silver in a soluble form to a suspension of bacteria which are actively oxidizing a sulphide mineral substrate containing no silver. The particles have also been observed forming on bacteria attached to the flat surface of silver-rich copper sulphide mineral (tetrahedrite) that had been introduced into a bacterial suspension growing on a sulphide mineral substrate free of silver. Attempts to obtain similar results with bacteria grown in the presence of ferrous sulphate alone have been unsuccessful but these silver sulphide particles

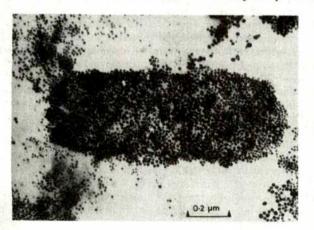


Fig. 1 Electron micrograph of a single bacterium emptied of its cell contents showing adhering silver sulphide particles, after recovery from a sulphide mineral leaching experiment in which the mineral contained trace quantities of silver.

can be formed on cells grown on elemental sulphur. Therefore, prerequisite for the formation of these particles is the presence of sulphide anions as well as silver cations. The reasons for the preferred nucleation of the silver sulphide on the bacterial wall are not clear but this preference is evident from the fact that in some experiments as much as 25% of the bacterial mass recovered after leaching of a silver sulphide mineral consisted of silver.

The finding reported here has interesting practical applications for the recovery of silver from sulphide ore minerals and is also of interest geologically, as it may indicate how silver was released, concentrated and relocated in surface ore deposits which previously had been affected by bacterial action.

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Mineralization of organic matter in the sea bed—the role of sulphate reduction

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The bacterial reduction of sulphate to sulphide at the sea bed is a key process in the oceanic sulphur cycle, and is responsible for the oxidation of organic matter which becomes buried below the oxic and sub-oxic zones of the sea bed. The oxic surface layer of the sea bed varies in thickness from a few millimetres in sheltered coastal areas to ≥1 m in pelagic sediments 1.2. Below this layer, organic matter is mineralized mainly by fermenting, denitrifying, sulphate-reducing and methane-producing bacteria. Sulphate reduction is the predominant terminal step in the mineralization processes of sulphate-rich shelf sediments where the sulphate reducers inhibit the methanogens by competing with them for common substrates3-5. Sulphate reduction may therefore have a quantitatively important role in the overall oxidation of organic matter in the sea bed. Recently, concurrent measurements of oxygen uptake and sulphate reduction in a coastal sediment have demonstrated the importance of the sulphate-reducing bacteria in the mineralization of organic carbon. I present here the first comparative survey of aerobic and anaerobic mineralization in the sea bed based on direct rate measurements of the two processes. The results demonstrate a surprisingly high contribution from the sulphatereducers. In coastal sediments, this specialized group of bacteria oxidized as much organic matter to CO2 as did all the aerobic organisms. Their relative contribution decreased three fold over the continental shelf from the shore to a depth of 200 m.

Oxygen consumption and sulphate reduction were measured in shelf sediments using previously described techniques7 Sediment cores were collected from 58 stations during cruises in Danish fjords and in Kattegat and Skagerrak, the two seas connecting the Baltic Sea with the North Sea. Measurements were made in the laboratory or onboard ship within 1 day after sampling. Sulphate reduction was analysed down to 15 cm depth in the sediment; measurements in cores of 2-3 m depth showed that most of the activity (75-95%) occurred in the topmost 0-15 cm (data not shown). Below 15 cm depth, I found significant (up to 40%) reduction only in cores from 200 m

Figure 1 shows the data obtained from all the sediment stations. The sediment types consist of a wide range of grain

sizes (from medium sand to clay) and organic content (0.7-18% dry weight). There is a clear correlation between the measured rates of oxygen consumption and sulphate reduction in these shelf sediments. This is partly a result of their mutual regulation by the influx of organic matter to the sea bed through sedimentation from the water column⁹⁻¹³. It is also due to the following inherent coupling between the two processes. During the bacterial reduction of sulphate, H₂S may be produced in large excess over the binding capacity of iron. The fraction of the H₂S which was precipitated by iron and converted into pyrite was calculated from the total (solid phase) reduced sulphur accumulation with depth and from the sedimentation rates (ref. 6 and B.B.J., unpublished results). The fraction varied from \sim 5% at 0-2 m depth to 10-20% at 20-200 m depth. Thus, an excess, corresponding to 80-95% of the H₂S produced, must have been re-oxidized back to sulphate at or near the sediment surface. The dark uptake of oxygen, which was the ultimate electron acceptor for the sulphide oxidation, therefore included 80-95% of the mineralization via sulphate.

This has important implications for the oxygen consumption of the coastal sea bed. The molar ratio of oxygen uptake to sulphate reduced averaged about 4:1 in sediments at 0-2 m and 2-20 m water depth (Fig. 1, broken line). As the oxidation capacity of 1 mol of sulphate is equivalent to 2 mol of oxygen, the ratio was 2:1 in terms of electron flow. Because 90-95% of the sulphide produced at 0-20 m depth was re-oxidized by oxygen, this means that $\sim 50\%$ of the oxygen uptake was

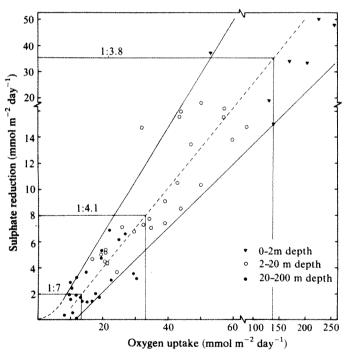


Fig. 1 Oxygen uptake and sulphate reduction rates (0-15 cm depth) of marine sediments from 0 to 200 m water depth in Kattegat, Skagerrak and Danish fjords. Sediments were carefully collected in 3-5 cm-wide coring tubes, which were kept at in situ temperatures under circulating, aerated water from the sampling locality and which preserved undisturbed the core stratification. Oxygen uptake was measured as the rate of disappearance of O2 from the water over stoppered sediment cores in the dark. Experiments were stopped after 10-20% O2 depletion to avoid decreasing uptake rates due to prolonged incubation. No bias in the O_2/SO_4^{2-} ratios due to pressure release is detectable for these sampling depths. The data are averages of cores for stirred and stagnant water columns. Sulphate reduction was measured by injecting 2 µl aliquots of ³⁵S-labelled sulphate into whole cores at 1-2 cm depth intervals. Labelled sulphide was analysed after a few hours' incubation. The vertical series of individual rates were integrated with depth to give the rate per area. Each data point represents 8-50 oxygen uptake and 20-100 sulphate reduction measurements per station. Details of the experimental techniques and of potential errors are discussed elsewhere'

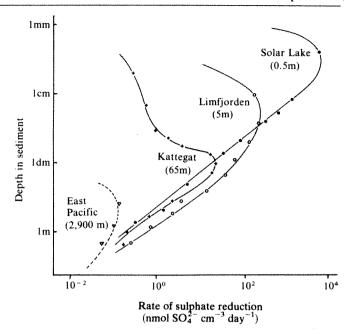


Fig. 2 Depth distribution of bacterial sulphate reduction rates for four sediment types. Water depths are indicated. Data are from Solar Lake¹⁴, Limfjorden⁶, Kattegat (B.B.J., unpublished results), and East Pacific¹⁵.

consumed by chemical or bacterial oxidation of the sulphide. The remaining 50% was used for respiration by the benthic fauna and by other aerobic organisms which use oxygen to oxidize directly the organic matter. In terms of oxidation of organic carbon, the contribution by the sulphate-reducing bacteria relative to the aerobic organisms was then $\sim 1:1$.

These conclusions generally hold for those sediments studied which have a community metabolism above $20\text{--}25 \text{ mmol } O_2 \text{m}^{-2} \text{ day}^{-1}$ (Fig. 1). Similar calculations made for sediments at 20--200 m water depth show an average O_2/SO_4^{2-} consumption ratio of 7:1 (Fig. 1). With 80% of the sulphide being re-oxidized by oxygen, $\sim 25\%$ of the oxygen uptake must have been consumed for H_2S oxidation. The mineralization of organic matter by sulphate reducers was thus equivalent to 35% of that directly mineralized by the aerobic organisms.

The relative importance of sulphate reduction in the shelf sediments investigated decreased with decreasing community metabolism and therefore with increasing water depth and distance from land. This may be explained by the fact that as the general metabolic rate of the sediments decreases the oxidized surface layer increases in thickness. The organic matter which reaches the deeper lying sulphide zone is therefore at a later stage of decomposition and relatively more refractory to further mineralization. The overall rate of mineralization which is expressed through the oxygen uptake consequently varies in direct proportion to the rate of organic sedimentation¹ whereas sulphate reduction varies rather in proportion to the square of the sedimentation rate 10,11. Once the organic matter has reached the sulphide zone, however, it has a long residence time and can sustain the low rate of sulphate reduction over great sediment depths. The range of sulphate reduction rates calculated per unit area of the sediment is therefore much less than the range of the peak rates. Figure 2 illustrates this trend in four sediment types which differ 100,000-fold in their peak rates of sulphate reduction. In the most dynamic sediment from a small salt pond in Sinai, Solar Lake, 90% of the entire sulphate reduction occurred within the uppermost 0.1-3 cm (ref. 14). With increasing water depth, the main activity was confined to deeper but thicker sediment layers of 1-10 cm in Limfjorden⁶ and 5-20 cm in Kattegat (B.B.J., unpublished results). In sediments of the East Pacific continental slope at 2,900 m water depth, sulphate reduction was traced from a few decimetres to several metres' depth¹⁵.

Sulphate reduction in the sea bed thus occurs mainly in the coastal zone. Shelf sediments, such as those studied here from the shoreline to 200 m depth, cover an area comprising only 8.6% of the world's ocean, but ≥90% of the oceanic sulphate reduction occurs here1:

The demonstrated importance of sulphate reduction in the overall mineralization of organic matter leads to a last essential point. The anaerobic bacterial food chain in which sulphate reduction is the terminal step, must be able to almost completely oxidize a wide range of organic compounds to CO2. Newly isolated strains of sulphate-reducing bacteria can oxidize all the major fermentation products (such as C-1-18 saturated fatty acids, lactate, ethanol, succinate, and benzoate, H2) completely to CO₂ and H₂O (ref. 16). Several of these strains have been found in high numbers in coastal marine sediments of the type studied here¹⁷. Furthermore, laboratory studies on similar sediments have shown that the lower fatty acids (acetate, propionate and butyrate) together with hydrogen³, are the main electron donors for the sulphate-reducing bacteria18. These results explain the discrepancy observed between the very restricted metabolic potential of sulphate-reducing bacteria which previously were known from marine environments (that is, Desulfovibrio spp.) and the evidence for an almost complete mineralization of organic matter via sulphate as demonstrated

This work was supported by grants from the Danish Natural Science Research Council. I thank Preben G. Sørensen for assistance during cruises and in the laboratory.

Received 14 December 1981; accepted 16 February 1982

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Ctenodactylid rodents in the Miocene Negev fauna of Israel

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We report here that the first rodents from the early (or early middle) Miocene of North Africa have been found in the Rotem and Yeroham Basins in the northern Negev of Israel. The rodents are associated with a large-mammal fauna resembling those of Gebel Zelten (Libya) and Moghara Wadi and Siwa Oasis (Egypt). These are the earliest records of Miocene rodents north of the Sahara, and strongly support reconstructions of a drier, more open environment in the northern part of Africa compared with the equatorial regions, with obvious significance for the evolution of grassland-adapted faunas.

As a result of two seasons (1979-80) of collecting, fossil rodents, primates and carnivores have been recovered in a new site ('Anthracothere Hill') in the northern Negev of Israel

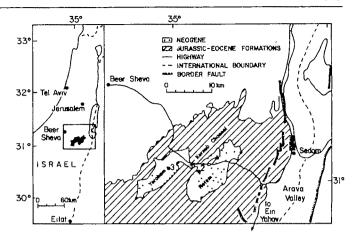


Fig. 1 Neogene basins of the northern Negev, Israel, -16-17 Myr (ref. 1).

(Fig. 1, site 1). Already these specimens have quadrupled the number of fossils known from the Negev; further study of Oron Road (site 2), which has been known for almost 30 yr, is just beginning.

Fossils of 'hippopotamus' and 'elephant' were first observed at Oron and Yeroham (site 3) during exploration for oil in the Negev during the 1950s². Savage and Tchernov³ identified these as an anthracothere and Mastodon (now Gomphotherium4) angustidens and Deinotherium bavaricum (now deinotherium hoblevi4), and also listed rhinocerotid, tragulid and hyaenodontid specimens. Stratigraphy of sites 2 and 3 was reviewed by Neev² and Savage³, sites 1 and 2 by Hoexter and Weinberger (in preparation) and sites 1-3 by J.A.V.C. Fossils from all three sites occur in basin-filling fluviatile deposits which have generally been correlated with the upper Mingar Member of the Hatseva Formation in the southern Jordan Rift Valley (Wadi Arava)⁵. We assume that deposition at these sites was approximately simultaneous, but because depositional environments are clearly different, the faunal lists are separated (Tables 1, 2).

In the Yeroham sites, specimens are found on the surface or in a poorly cemented, friable sandy matrix in low-relief badlands and gullies. An oyster reef (Crassostrea) 5 m above the mammalian fossils represents the maximum onlap of a marine transgression. The vertebrate fauna is depauperate, consisting of only four species (Table 1) represented by 22 specimens. Their size and shape appear to match the selection seen in Behrensmeyer-Voorhies Group III (ref. 6): a small collection of dense bones and teeth accumulated in a lag deposit.

At the Oron Road site, fossils occur just below the ground surface on the south-west-facing slope of the Rotem central arch, ~30 m south of the road. The matrix is indurated conglomerate with occasional pockets of quartzose sand. Clay balls, silt, flint cobbles and Cretaceous invertebrates from the flint occur rarely. Rare fragments of Crassostrea (Z. Lewy, personal communication) indicate either drainage from the Yeroham Basin, the reverse of early middle Miocene flow⁵, or the former presence of oyster reefs in eroded and redeposited parts of the Rotem sediments. Small fossils form 7% of a total of 73 specimens; no fish spines have been recovered. Large mammal bones and teeth were abraded in transport and subject to bioturbation,

Table 1 Yeroham Basin fauna

Pisces Lates sp Vertebrae Reptilia Crocodilia Crocodylus ?lloydi Scute, pubis, teeth Mammalia Pelves Proboscidea, indet. Gomphotherium angustidens 1 Molar fragments, M. Prodeinotherium hoblevi* Mandable, molar crowns

sp., Species unknown; indet., indeterminable Ref. 3 (in part).

desiccation and fracture *in situ*. The assemblage is Behrensmeyer-Voorhies I-II-III, redeposited after bulk transport in flood conditions⁶.

At Anthracothere Hill fossils occur on the steeply inclined eastern face of a low ridge, Giv'at Kadmai. A few large fossils, for example, a rhinoceros phalange and a palm stipe, were recovered in the slope wash. Strata underlying this ridge, not fully revealed, include 7-10 m mudstone; 1-1.5 m of fossiliferous, loosely indurated red-yellow quartzose sandstone and pebbly grit in 1-2-cm layers, interbedded with massive white sandstone; and 1-8 m of coarse sand, locally crossbedded; the ground surface is Cretaceous flint cobbles. Baculites, Ostrea, Dentalium and Exogyra fragments from Cretaceous flint are rare; chalcedony cobbles to 0.5 m, calcareous pebbles, limonite nodules, clay balls to 0.2 m and silt are randomly interspersed. Sand grain analyses and upward fining of the layers indicate a high-energy palaeoenvironment, such as a river bank or channel deposit.

Small fossils, including fish bones and spines, vertebrae and teeth of small mammals, are about one-third of 445 specimens from Anthracothere Hill (Table 2). Large bones, 10–20 cm, well preserved as to detail but not robust, such as a juvenile rhinoceros mandible, a bovid tibia and several ribs, were found encased in grit. These may have been scavenged or fresh carcasses cached by crocodiles or other predators⁶. The palate of a very young post-hatchling crocodile found within one such grit layer is consistent with underwater burial. Bone pebbles 0.2–10 cm occur, but overall the large numbers and well preserved aspect of the small fossils suggest Behrensmeyer-Voorhies class I–II and deposition after brief transport.

Vertebrate microfossils in fine white sand, a palm trunk and root casts have been found in Kurnub Channel, the Miocene channel between Rotem and Yeroham Basins (Fig. 1). A humerus of the Oligocene-Miocene dugongid Halitherium was recently found at Ein-Yahav in the Arava. In sum, despite variations in abrasion, taphonomy and stratigraphical context, it is probable that the fossils of Yeroham, Oron and Anthracothere Hill were deposited at more or less the same

time, perhaps within a few tens of thousands of years of one another. Although direct dating of the fossils has not yet been obtained, if the strata in these basins belong to the Hatseva Formation, they are older than the faulting that now separates the basins from the type area in the Jordan Rift; the age of this rifting has been estimated from fission-track analysis of the 'Mottled Zone' at 13.6 ± 2.0 Myr (refs 8, 9). On the other hand, the marine transgression and aggradational episode to which the Yeroham oyster reef may be related, may be either that of the late early Miocene 'Vindobonian transgression' associated with the guide fossils Orbulina and Borelis melo curdica at about 15-16 Myr, which in Israel was preceded by an erosional phase and followed by a desiccation event¹⁰, or the reef-building episode in the upper Ziqim Formation of late Miocene age, ~9 Myr (ref. 11). The mammal fauna, although not highly age-diagnostic in itself, seems clearly to be of earlier rather than later Miocene age³ and similar to later early Miocene faunas of Gebel Zelten, Wadi Moghara and Siwa Oasis, as is shown below.

Two taxa of 25, Dicerorhinus and Tomistoma, are found only at Oron; four taxa of 28, bird, insectivore, primate and cricedontid rodent, are found only at Anthracothere Hill, but these differences reflect sampling intensity and taphonomy. If palaeoecology can be inferred from similar modern forms^{12,13}, the combined inventory represents five habitats: brackish lagoon (dugong, sharks); river/lake/swamp (anthracothere, crocodiles, trionychid turtle, fish, palms); forest (testudinid turtle, tragulid, proboscideans, primate, creodont, carnivore); grassland (giraffid, bovid, rhino, creodont, carnivore, cricetid and pedetid rodents); and semi-arid to rocky landscape (gazelle, ctenodactylid rodent). Continued sampling may change these econiche estimates, but the impression is a large number of habitats confined in a small terrain, and lake basins draining a few hundreds of kilometres and debouching on to a coastal corridor.

The list contains 27 African endemic taxa and what appears to be an immigrant, the ctenodactylid *Metasayimys*, represented by 12 incisors from the Rotem sites. *Metasayimys* in the Rotem

	Table 2 Or	on and Anthracothere Hill fauna	
		Oron Road	Anthracothere Hill
Pisces		Tr. A.	T4L
Chondrichthyes	indet.	Teeth	Teeth
Osteichthyes	indet.	77 . A. 3	Dentary
Reptilia	Lates sp.	Vertebra	Vertebrae, spines, preoperculum
Chelonia	Trionyx sp.	Scutes	Scutes
Cheloma	Testudinidae	Scutes	Scutes
I acertilia	indet.	Vertebra	Vertebrae
Crocodilia	Crocodylus ?lloydi	Scutes, teeth, mandible	Scutes, teeth, mandible
Crocoma	Tomistomidae	Teeth	Scarce, teeth, manuale
Aves	7Passeriformes sp.	reem	Ulnar shaft
Mammalia	it assemormes sp.		Omai shart
Insectivora	indet.		Mandible
Primates	⁹ Cercopithecidae		Teeth, bone
Rodentia	Megapedetes sp.	Incisors	Incisors
Rodelida	Metasayımys sp.	Incisor	Incisors
	Cricetodon sp.	Thereof	Incisors, mandible, molar
Creodonta	indet.	*Tooth	Mandible
Carnivora	indet.	Phalange	Phalanges
Carmivora	Viverridae	rnatange	Humerus
	Canidae		Humerus
Perissodactyla	Camaae		riumei us
Rhinocerotidae	indet.	*Teeth, metapodial, tibia	Humerus, phalanges, mandible
ramioceronane	Dicerorhinus	Cubitus, humerus	ramorus, pantingos, materiore
Artiodactyla	Dictioninas	Cubitas, numerus	
Anthracotheriidae	indet.	*Teeth	
	?Hyoboops	Premolar	Premolar
Suidae	indet.	Molar	Teeth, phalanges
Tragulidae	indet.	*Tooth	
	Dorcatherium sp	Caput femoris	Teeth, calcanei
Gıraffidae	indet.	Molar	P^2
Bovidae	Boselaphini, indet.	Tibia, calcanei, phalange	Teeth, astragulus, sacrum, tibia
	Eotragus sp.	Molar	Mandibles, horn, teeth, M1
	Gazella sp.	Mandible	Calcanei
Proboscidea	indet.	Vertebra	Vertebrae
	Gomphotherium angustidens	Molar fragments, phalange	Molar fragments
	Prodeinotherium hoblevi	Phalange	Molar, deciduous premolar

For abbreviations see Table 1 legend.

Table 3 Negev genera: correlation with fauna from other African and European sites

	Rusinga early Miocene ~-18 Myr	Gebel Zelten ⁹ early middle Mioœne ~-16 Myr	Negev	Europe MN 4 ~-16-17 Myr
Metasayımys			+	[+]
Megapedetes	+		+	(+)
Dorcatherlum	+	+	+	+
Gomphotherium	+	+	+	+
Prodemotherium	+	+	+	+
Hyoboops	+	+	+	
Eotragus	?	+	+	+
Gazella	?	+	+	
Dicerorhinus	+		+	

Sources Rusinga, Kenya, ref. 23, Appendix; Gebel Zeiten, Libya, ref. 28, Table 2; Negev, Israel, this work; Europe, refs 14, 26 Table; [] ctenodactylid sp., Pasalar, Turkey, ref. 15; () Bayraktepe, Turkey, ref. 24, possibly younger,

sediments may be the earliest African appearance, if this fauna proves to correlate with MN zone 4 (ref. 14, see below). The next youngest record of this group is 'Ctenodactylid, sp. indet.' at Pasalar, Turkey¹⁵, in zone MN 5 and the equivalent, or slightly younger, Metasayimys cf. intermedius of the Dam Fm, As Sarrar, Saudi Arabia (H. Thomas, personal communication). Related species are *Metasayimys intermedius* Sen and Thomas 16 of the Hofuf Fm, Hasa Province, Arabia, "older than ... Beni Mellal (our translation)"; Metasayimys jebeli Lavocat17 of Beni Mellal, Morocco, and Sayimys from the Chinji Fm, lower Siwaliks, India 18,19, also about this age.

Hyoboops and Dicerorhinus have been considered slightly earlier immigrants from Asia, observed at Dera Bugti, Pakistan20-22, but as both appear at the approximately contemporaneous early Miocene sites in Kenya²³, an African origin seems equally probable. The creodonts and carnivores of Rotem-Yeroham (in preparation) also have African early Miocene affinities. African emigrants into Eurasia in the early Miocene are Dorcatherium, Gomphotherium, Prodeinotherium and Eotragus. Megapedetes, also emigrant, has been recovered at Bayraktepe, the southern Dardanelles, Turkey24, with a mix of faunal elements, some as young as ~13 Myr (for example,

To determine biogeographic correlation of the Negev fauna, we relied on radiometric- and marine-controlled dating of Rusinga²⁵, Europe^{14,26}, the Mediterranean²⁷ and a transect of the Israel shoreline 10. The major datum points are the Rusinga lavas at -18 Myr and the appearance of the foraminifer Orbulina suturalis at -16 Myr; Gebel Zelten is set at -16 Myr.

In Table 3, based on small-to-large herbivores, we equate the Negev basins and Gebel Zelten, Libya, and by extension, Moghara and Siwa, Egypt²⁸⁻³⁰. (Determination of the Rotem creodonts and carnivores may alter the estimate.) The Rotem-Yeroham assemblage seems to be slightly older than that of As Sarrar in western Arabia (H. Thomas, personal communication) and ranked by age, older than the fauna of Al Jadidah and Ab Dabtiyah, Saudi Arabia, the India-Pakistan sites, and Fort Ternan, Kenya, the last dated 14 Myr (refs 31-35). Hyoboops and Dicerorhinus suggest that Rotem-Yeroham is younger than Dera Bugti^{21,22} and Rusinga²³.

The best estimate for the Negev fauna is thus -16-17 Myr, a time of global warming and land emergence observed in the Mediterranean Sea and at continental margins 10,36,37. The thermal high that began in the earliest Miocene was just at peak and Israel shore emergence was maximal. Cogley's reconstruction of the eastern proto-Mediterranean -20 Myr (J. G. Cogley, personal communication) shows a filling-in between Israel and Turkey which agrees with the ctenodactylid appearance at Pasalar. Except for the faunal comparisons, however, the early middle Miocene has revealed no compelling geological evidence for a connection between Negev basins and Europe via Turkey.

We thank P. Andrews and F. Clark Howell for comments on an early draft, C. J. Cogley and H. Thomas for permission

to quote from unpublished manuscripts, and the Friends of Anthracothere Hill and the L. S. B. Leakey Foundation and the Energy Ministry, the State of Israel, for their support. We also thank H. de Bruijn, M. Gayet, P. H. Greenwood, S. Gross, G. Gvirtzman, J.-J. Jaeger, J. Lorch, Z. Lewy, C. Madden, D. Neev and S. Sen for review and discussion of our conclusions.

Received 31 December 1981; accepted 16 February 1982

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Free Ca2+ and cytoplasmic streaming in the alga *Chara*

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Plant cells, like those of animals, contain the Ca2+-binding protein calmodulin¹⁻⁴. By analogy with animal cells it has thus been suggested that the intracellular free Ca2+ concentration may have an important role in the regulation of plant cell activities. This suggestions has been supported by various physiological experiments, but so far direct evidence, invol ving measurements of intracellular Ca2+ levels, has not been obtained. We describe here measurements of intracellular Ca2 in the giant alga Chara by microinjection of the protein aequorin, which emits blue light in proportion to Ca2+ concentration. Chara exhibit an ATP-dependent cytoplasmic streaming shown to be inhibited by Ca^{2+} (refs 5, 6). We report that *Chara* cells have a low free Ca^{2+} concentration, comparable with those of animal cells, and that action potentials which inhibit cytoplasmic streaming⁷ increase this Ca²⁺ concentration substantially.

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Aequorin was injected into internodal cells of Chara corallina or Nitella sp., and initially showed a high light emission that, except for occasional transient increases, declined rapidly for 30-50 min (Fig. 1a). This probably reflected a relatively high cytoplasmic free Ca^{2+} concentration ($Ca_{\rm cyt}^{2+}$) as a result of the injection process, and precluded any detailed interpretation. In the next phase the light signal was low, subsequently declined very slowly and showed no transient increases in an undisturbed, undamaged cell (Fig. 1a). This is consistent with the injected aequorin now being evenly distributed by cytoplasmic streaming and with $Ca_{\rm cyt}^{2+}$ having declined to a low value, and this conclusion is supported by the finding that cells lysed in a solution containing a Ca^{2+} saturating for aequorin (solid line, Fig. 1b) contained active aequorin for up to 14.5 h after injection.

The peak lysis signal after correction for poor mixing $(L_{max}$ in saturating Ca^{2+}) and the *in vivo* aequorin signal (L) in Ca^{2+}_{cyt} were used to determine Ca^{2+}_{cyt} from a calibration curve relating L/L_{max} to free Ca^{2+} in vitro (Fig. 1b,c). The solutions used for this curve resembled characean cytoplasm in pH and free Mg^{2+} (Fig. 1c). Here two values for free Mg^{2+} were used because of the range reported for ATP, probably the major Mg^{2+} chelator. From the 3 mM free Mg^{2+} curve, the mean Ca^{2+}_{cyt} was found to be 0.22 μ M for Chara (range 0.1–0.56 μ M, n=6) and 1.1 μ M for Nitella (range 0.79–1.6 μ M, n=4). At 1 mM free Mg^{2+} , the mean values were lower, that is, 0.1 μ M and 0.44 μ M, respectively. Cells appeared undamaged by several physiological criteria and neither chloroplast light absorption nor partial aequorin inactivation by enzyme inhibitors generated at cell lysis introduced major inaccuracies into the estimates of Ca^{2+}_{cyt} . In addition, these values agree with those suggested by cell perfusion experiments 5.6.8.9.

Uncentrifuged cells were also injected with aequorin using a large tipped micropipette which penetrated the vacuole through ~10 μm of parietal cytoplasm¹0. The resulting relatively rapid light emission left little or no unreacted aequorir detectable on cell lysis after 15 min while unreacted aequorir was present 14.5 h after injection into the cytoplasmic compartment. The half time for aequorin utilization in a medium of sap expelled from cut cells indicated a near saturating Ca²-(L/L_max = 0.56). Any vacuolar inhibitors, including low vacuolar pH (Fig. 1 legend and ref. 11) will make this ar underestimate of vacuolar Ca²-, suggesting that the free Ca²- is >10⁻⁴ M in this compartment.

Thus characean cells contain a cytoplasmic compartmen where free Ca^{2+} is maintained at a concentration of micromolal or less and a vacuolar compartment where the free Ca^{2+} is $>10^{-4}\,\text{M}$. These measurements of free Ca^{2+} should be compared with measurements of total Ca^{2+} for characean cells indicating 3–12 mM in the vacuole 12–15 and 2–8 mM in the cytoplasm 6.15.

On electrical or mechanical stimulation, characean cells propagate action potentials along both the plasma and tonoplass membranes¹⁰ and this is associated with streaming cessation⁸. The ability of elevated Ca^{2+} concentration to inhibit both streaming in perfused characean cells^{5,6,16}, as well as chloroplass rotation in cytoplasmic fragments¹⁶ isolated from characean cells, makes a rise in Ca_{crt}^{2+} a likely explanation for streaming cessation by the action potential in intact cells⁸.

Chara cells with cytoplasmic aequorin were stimulated with external electrodes and changes in vacuolar potential (Ψ_{vo}) and either aequorin light emission or streaming velocity were measured. The electrical transient was accompanied by a large

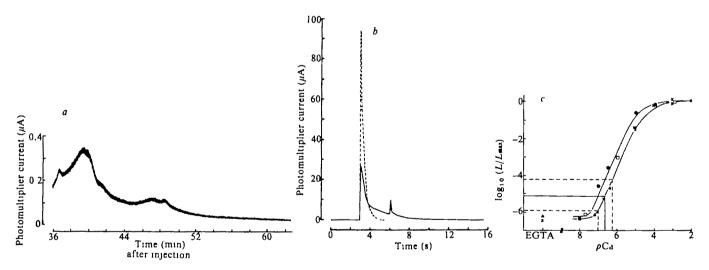


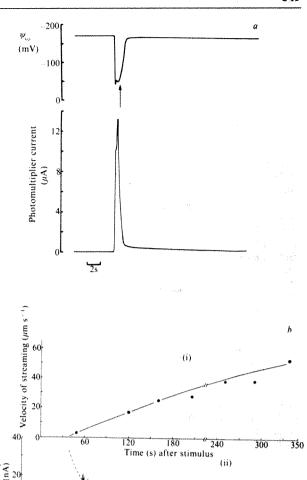
Fig. 1 Acquorin prepared as in ref. 35 was dissolved in (mM): 150 KCl, 10 N-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid (Na₂TES), pH 7.5, and ~50 nl (10% cytoplasmic vol) was injected into an internodal cell (~40 mm long) of Chara corallina or Nitella sp, which had been centrifuged at low speed for several minutes, and immersed, to reduce turgor, in (mM): 0.1 KCl, 1.0 NaCl, 200 or 250 sucrose, with the centrifugal end exposed. Acquorin was injected into the resulting endoplasm plug, the pipette withdrawn, and the cell returned to full turgor by diluting out sucrose with artificial pond water (APW) (mM): 0.1 KCl, 1.0 NaCl, 0.1 CaCl₂, 2 Na₂TES, pH 7.2). Acquorin light emission was detected with an EMI 9635A (~900 or ~1400 V) photomultiplier (signal output to a chart recorder via current-voltage convertor (RC = 200 ms or 0.1 ms) protected from amblent light by a shutter; (bar on time axes denotes shutter closure). All photomultiplier currents were standardized by conversion to their equivalent current at ~1,400 V with photomultiplier dark current (4.5 nA) and chamber ambient light (shutter open, unmjected cell, ~0.5 nA) subtracted. a, Acquorin light emission from cytoplasmically injected Chara cell. b, Light emission (solid line) following crushing of similar cell m a lyst solution (mM): 10 CaCl₂, 50 Na₂TES, 1% (v/v) Triton X-100, pH 7.3. Rapid, complete acquorin discharge required both crushing and detergent. L_{max} for Ca²/₂ calculation (ref. 45 and fig. 1c) requires correction for poor mixing as in the broken curve enclosing same area and with 1/1/2 = 0.49 s (1/2) measured in vitro when acquorin is rapidly mixed with lysis solution at ~20 °C (see ref. 35). c, In vitro cabbration curves for measuring Ch²/₂ tron L/L_{max} and L/L_{max} and Macla Ch²/₂ to 1.0 or 3 (×, A) free Mg²⁺, 30 Na₂TES, pH 7.6 and various Ca²⁺ concentrations achieved below 10⁻⁴ with EGTA. Mg²⁺ concentration approximated to cytoplasmic free Mg²⁺ calculated as total free Mg²⁺ (3.6 mM; ref. 5) minus

increase in light emission (Fig. 2a) which was not seen if uninjected cells were used. As in uninjected cells, the detailed shape of the electrical record varied from cell to cell, and to some extent with time in an individual cell, and the light emission varied similarly. Action potentials, which were all-ornone and typical of the characae, could be elicited repeatedly over several hours with only a gradual decline in the amplitude of the aequorin signal resulting from an estimated 4% of the aequorin being consumed by each action potential response (Fig. 2 legend). The peak light emission corresponds closely to the visually estimated time for streaming cessation (Fig. 2a; see also ref. 17). However, aequorin light returns to its resting level in 40-60 s, while streaming in different cells may take 75-360 s and have only reached some 25% of its full rate by the time aequorin light has fully recovered (Fig. 2b). If aequorin light accurately reflects changes in Ca_{cyt}²⁺, then a hypothesis of Ca²⁺ control requires a mechanism in which there is rapid inhibition by elevated Ca²⁺_{cyt} but slow recovery extending many seconds beyond the recovery time for Ca_{cyt}^{2+} . In vertebrate smooth muscle, where the time course of Ca_{cyt}^{2+} and tension also diverge¹⁸, free Ca²⁺ is thought to exert control via a calmodulindependent myosin light-chain kinase, causing phosphorylation which, in turn, is regulated by a cyclic AMP-dependent followed eventually by a slow dephosphorylation step¹⁹⁻²⁴. The existence of a similarly complex regulatory system in Chara may generate the substantial divergence observed between the recovery of streaming and of aequorin light.

We can also determine the magnitude of the free Ca2+ change as a result of the action potential. Ca2+ was calculated to rise 30-fold to 6.7 µM (mean of 6 cells) during a Chara action potential (from Fig. 1c taking $L \propto [Ca]^2$) (42-fold for Nitella to 43 μ M, n = 4). This would take Ca_{cyt}²⁺ into the range where ATP-dependent streaming in perfused cells is strongly inhibited by Ca²⁺ (refs 5, 6, 25) but it is still less than the 10⁻³ M Ca²⁺ needed to stop streaming completely in perfused cells⁸, as happens at the action potential¹⁷. This discrepancy implies (1) that there is another inhibitor of streaming associated with the action potential (perhaps, a change in cytoplasmic pH): (2) that the cell model is depleted by perfusion of some as yet uncharacterized regulatory protein, and is thus less sensitive to Ca2+ than the intact cell; or (3) that the peak Cacyt has been underestimated. This would occur if aequorin light emission were inhibited by, for example, a rise in the concentration of H⁺ or free Mg²⁺, if Ca²⁺_{cyt} did not increase in all parts of the cytoplasm, or if it increased asynchronously in different regions (see, however, Fig. 2 legend). The return of aequorin light to precisely its pre-stimulus value (Fig. 2b) indicates, however, that if any ionic changes affecting the sensitivity of aequorin occur, they must be short-lived.

Replacing external Ca2+ with Mg2+ leads to a reversible loss of excitability in Chara which may be due to a specific requirement for Ca²⁺ to initiate the transient change in anion permeability26. Mg2+ treatment of cells containing cytoplasmic aequorin led to partial depolarization and to a reversible decline in the magnitude of both the electrical and aequorin-light transients (Fig. 3a). This was accompanied by a loss of streaming inhibition which returned with the electrical and aequorin transients when Ca²⁺ was restored to the bathing solution. There appeared to be a degree of separation between changes in the electrical transient and the changes in the aequorin light response (Fig. 3a), with the alterations in electrical responsiveness preceding the changes in aequorin light emission during both the loss and recovery phases. The partial recovery of the electrical transient before the light transient is consistent with the idea that other ions^{14,26,27} besides Ca²⁺ have a role in the electrical transient besides Ca²⁺ have a role in the electrical transient. In Nitella, complete replacement of Ca2+ by Mg2+ results in no loss of excitability, a prolongation of the electrical response and no loss of streaming²⁸, suggesting that cytoplasmic Ca² stores are less likely to be a source of the changes in Ca_{cyt}²⁺: the results in Fig. 3a also support this notion, as do tracer influx experiments⁸

One batch of Nitella did not show the typical characean



Time (s) after stimulus

(iii)

Fig. 2 a, Changes in electrical potential (Ψ_{vo}) and aequorin light emission during an action potential. The 'tail' to the electrical record indicates that the electrode tip is in the vacuole10. Current pulses from a Devices Stimulator were applied to cell via full length external Ag/AgCl wire electrodes immersed in pools of APW, separated by a silicone grease barrier. The microelectrode signal was fed to a Keithley electrometer and then to a Tekman TE220 chart recorder. Either aequorin light emission, recorded from 90% of the cell length by a standard photomultiplier arrangement, or cytoplasmic streaming measured close to the microelectrode, were determined. Streaming cessation (determined visually) occurred ~300 ms after maximum depolarization (arrow). Calculations indicated that 3.7% (n=4) of the injected aequorin is consumed during each action potential. b, Recovery of aequorin light emission (ii) to pre-stimulus level following an action potential indicated on the record of vacuolar potential (Ψ_{vo}) (iii). (Output from photomultiplier was shorted out during the stimulus to show recording at high gain during the recovery phase). The photomultiplier current includes resting light from injected cell (~2 nA) (see Fig. 1 legend). (i) Indicates that the recovery of streaming velocity to its pre-stimulus value of $\sim 50~\mu m \, s^{-1}$ was much slower than recovery of the aequorin light emission. Observations are from the same cell. T, 20 °C. The cessation of chloroplast rotation at the action potential8 indicated that motility inhibition occurred at all depths in the endoplasm. However, the inhibition spreads radially inwards through the endoplasm at 15 μ m s⁻¹ so that, if the increase is being observed, all regions will be affected but not synchronously. Radial spread through a typical thickness of endoplasm would take ~0.5 s (ref. 10). However, the time between the stimulus artefact and the membrane response recorded at the further end of the cell suggest that the cell was excited almost simultaneously by the extensive external electrodes. While some asynchrony and therefore underestimation of the peak Ca_{ev}²⁺ seems possible, the consumption of only 4% of the total acquorin during each action potential severely restricts the time for which the 10⁻³ M Ca_{est}²⁺ required for complete inhibition in perfused cells could exist. As $t_{1/2}$ for aequorin reaction is ~ 0.5 s at 10^{-3} M Ca²⁺, 4% of the aequorin would be consumed in 40 ms if this concentration was reached instantaneously. If allowance is also made for acquorin consumption during the finite rising and recovery periods, the existence of 10^{-3} M Ca²⁺ would have to be even briefer.

Electrical potential (mV) Photomultiplier current

100 -50

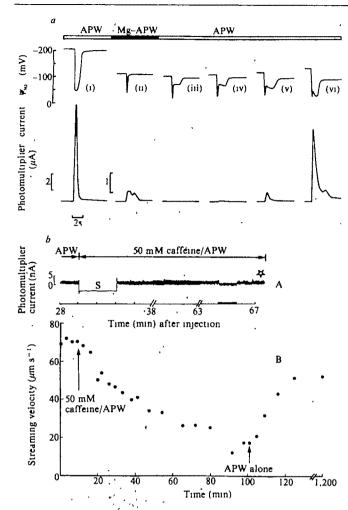


Fig. 3 a, Traces of vacuolar electrical potential (4,,) and aequorin light from Chara cell exposed for 26 min to Mg-APW (mM) 10 MgCl₂, 1 NaCl, 0.2 KCl, 2 Na TES, pH 7.2. Note that the electrical transient is preceded by a stimulus artefact clearly seen in trace (li) when the transient has been abolished. (ii) Mg-APW led to loss of electrical response and decline in acquorin light response that was complete by the time APW was restored (trace (iii)) Recovery of the electrical and aequorin light response (traces iii-vi) documented 13, 15, 24 and 40 mm after return of APW showed recovery of the aequorin light response to be slower than the electrical response as substantial electrical transients were observed in APW before a significant recovery of aequorin light response. Inhibition of streaming on stimulation was lost in Mg-APW, returning only slowly in APW. After 24 mm APW (trace v) there was no inhibition 30 s after stimulation, after 50 min in APW, streaming inhibition was complete (trace very similar to trace vi) but recovery was more rapid than normal (~75 s, see Fig. 2b) b, Inhibition of cytoplasmic streaming (B) in Nitella by 50 mM caffeine-APW was accompanied by no appreciable increase in acquorin light emission (A). The streaming record is from an uninjected cell but examination of the injected cell at the end of the light emission trace (4) revealed inhibited streaming which recovered on replacing the caffeine with APW. The artefact (S) on trace A represents a solution change with the photomultiplier output shorted out.

all-or-none behaviour¹⁰. Here stimuli of increasing magnitude evoked aequorin light transients of increasing size and resulted in correspondingly more marked inhibitions of cytoplasmic streaming^{29,30}. These results suggest that cytoplasmic streaming responds to Ca²⁺_{crt} levels intermediate between those found in the resting cell and those achieved at the normal action potential, behaviour which is qualitatively consistent with the response of perfused cells to such variations in free Ca²⁺ (refs 5, 6, 25).

Finally, Nitella cells were exposed to caffeine, which in muscle is able to release Ca²⁺ from the sarcoplasmic reticulum and cause contraction³¹. In plants, caffeine inhibits cytokinesis³², vesicle secretion³³ and chloroplast rotation³⁴. Caffeine inhibited cytoplasmic streaming in Nitella (Fig. 3b), although only at the rather high concentrations needed to stop chloroplast rotation in Coleochaete³⁴. However, the inhibition of streaming in Nitella

was not accompanied by any substantial increase in aequorin light emission (Fig. 3b). Thus caffeine inhibits a Ca² plant process, but apparently not by appreciably increasing

Ca_{cyt}²⁺ in the two characean algae studied is some 10⁴ times lower than total cytoplasmic Ca²⁺. While clearly an extrapolation, it seems likely that all plant cells will conform to this low Ca²⁺ pattern as have the diverse animal cells studied to date³⁵.

Given these low Ca²⁺ values, the external 10⁻⁴ M Ca²⁺ and 10⁻⁴ M Ca²⁺ in the vacuole provide large electrochemical gradients for Ca²⁺ entry into the cytoplasm at both the plasma membrane (cytoplasm ~170 mV negative) and the tonoplast (cytoplasm ~20 mV negative¹⁰). Although the measured Ca²⁺ influx at the plasma membrane is small ($\sim 0.04~\mathrm{pmol~cm^{-2}~s^{-1}})^{8.13}$, in isolation this would raise $\mathrm{Ca_{cyt}^{2+}}$ at the rate of $0.04~\mu\mathrm{M~s^{-1}}$, a large change in relation to the resting Ca²⁺; this calculation assumes a 10 μm-thick layer of cytoplasm and no binding or sequestration. Our results indicate that sequestering by cytoplasmic organelles may provide short-term control of Ca²⁺_{cyt}. Long-term stability would require Ca²⁺ movement either directly out of the cytoplasm across the plasma membrane or into the large vacuolar reservoir, presumably ultimately effluxing to the external medium; a flux of $0.04 \text{ pmol cm}^{-2} \text{ s}^{-1}$ would increase the total Ca²⁺ by ~1 mM in 7 h. In a situation where several organelles plus the plasma membrane and tonoplast may contribute to maintaining Ca²⁺_{cyt} the quantitative importance of each component process in vivo is at present difficult to predict.

The range of Ca²⁺_{crt} changes reported here corresponds well to the operating range for calmodulin and other Ca2+-regulated proteins⁵⁰ and suggests that changes in Ca²⁺_{crt} could regulate, either directly or indirectly, many aspects of plant cell biology. So far the action potential is the only proven effector of a change in plant Ca_{cyt}^{2+} and it remains to be determined whether action potentials in other plant cells^{36,37} use Ca_{cyt}^{2+} to exert their postulated effects on processes such as morphogenesis 38,39 Similarly, cytoplasmic streaming is the only physiological process known to be affected by that Ca²⁺_{cyt} change, although other Ca²⁺ effects have been postulated for characean cells, such as the possibility that light and plant hormones are also able to change Ca_{cyt} (Refs 40-44). It is now possible with this method to investigate these suggestions more precisely.

We thank Drs E. McRobbie, T. J. Lea & M. Beilby for useful discussion, R.E.W. received financial support from La Trobe University and the Australian Research Grants Committee.

Received 25 September 1981; accepted 8 March, 1982

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Non-coated membrane invaginations are involved in binding and internalization of cholera and tetanus toxins

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The binding of various biologically significant macromolecules to specific cell surface receptors is followed by their internalization, a process called receptor-mediated endocytosis1... In most cases, it has been shown that receptor-bound ligands cluster in characteristic, bristle-coated indentations of the cell surface known as coated pits1-3. In addition to coated pits, cultured cells have a population of smaller, non-coated membrane invaginations⁴⁻⁶, which may have a role in endocytosis^{2.7}. However, no receptor-bound biologically active ligand has been shown to enter cells via these non-coated invaginations. The studies implicating coated pits in receptor-mediated endocytosis concern essentially ligands that bind to receptors thought to be glycoproteins. We have therefore investigated by electron microscopy the endocytosis of cholera toxin and tetanus toxin, ligands which bind to membrane glycolipids, in particular to either GM₁ monosialoganglioside⁸⁻¹¹ or di- and trisialogangliosides12-15 respectively. We show here that in cultured liver cells, non-coated membrane microinvaginations are preferentially involved in both the initial binding and subsequent internalization of colloidal gold-labelled cholera and tetanus toxin.

The rat liver cell line KLTRYPV16 (provided by Dr G. Vergani) was maintained in monolayer culture as described elsewhere17. The cells were incubated with gold-labelled toxins as described in Fig. 1 legend, then washed three times with ice-cold phosphate-buffered saline (PBS) and either immediately fixed at 2 °C in 2% glutaraldehyde in PBS for 15 min, or warmed to 37 °C for 10 or 30 min before fixation. The cultures were further processed in situ for electron microscopy, as described previously18

The distribution of cholera toxin-gold and tetanus toxin-gold complexes at 2 °C on thin sections cut perpendicularly to the plane of the monolayer was essentially similar. Gold particles were scattered at irregular intervals along the plasma membrane, including the microvilli, but were frequently associated with non-coated, flask-shaped microinvaginations (Fig. 1a, b). In contrast, coated pits were labelled only very rarely. The association of toxin-gold complexes with the small, non-coated invaginations was also apparent in sections parallel to the substratum, in regions where the membrane had been cut tangentially (Fig. 1c, d). To check the specificity of labelling, cells

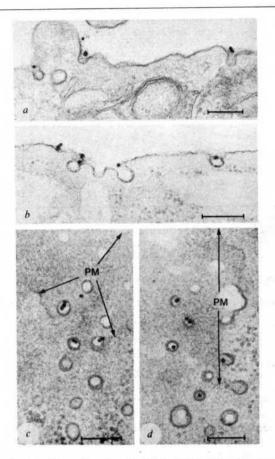


Fig. 1 Labelling pattern in cultured liver cells exposed to cholera toxin-gold (a, c) or tetanus toxin-gold (b, d) complexes at 2 °C. The preferential labelling of non-coated, flask-shaped microinvaginations is evident in sections cut either perpendicularly (a, b) or tangentially (c, d) to the plasma membrane (PM). As seen in a and b, gold particles are mostly located at the neck of the microinvaginations in these conditions. Colloidal gold (particle diameter ~15 nm) was prepared according to Frens²⁸ and the pH adjusted to 6.9 with 0.2 M K2CO3. Cholera toxin (Schwarz/Mann, New York) and tetanus toxin (given by Dr B. Bizzinni) were brought to 5 mM NaCl by passage through a Sephadex G-25 column (0.9 × 10 cm) equilibrated and eluted with 5 mM NaCl. The minimum amount of cholera toxin or tetanus toxin needed to stabilize a given volume of colloidal gold was estimated according to Roth and Binder²⁹. The crude toxin-colloidal gold preparations were centrifuged at 60,000g at 4 °C for 1 h and resuspended in phosphate-buffered (50 mM, pH 7.4) isotonic saline to a final concentration of 100 µg cholera toxin per ml or 1 mg tetanus toxin per ml. Cells grown to confluency in Linbro multi-well plates (Flow) were washed three times with ice-cold PBS and equilibrated at 2 °C for 5 min before incubation with cholera toxin-gold or tetanus toxin-gold at 2 °C for 30 min. Scale bar, 0.2 μm.

were first incubated at 2 °C for 30 min with either unlabelled cholera toxin (500 µg ml⁻¹) or tetanus toxin (2.5 mg ml⁻¹) in PBS, then exposed to the toxin-gold complexes. In such conditions, surface labelling was virtually absent.

In liver cells warmed to 37 °C for 10 or 30 min before fixation, gold particles were found in small non-coated cytoplasmic vesicles (Fig. 2a), in larger vacuoles having the morphology of multivesicular bodies (Fig. 2b) and in typical lysosomes. Coated pits or vesicles were only rarely labelled. To ensure that coated pits were functional in these cells, experiments similar to those described above were done after indirect labelling of concanavalin A (Con A) binding sites with Con A followed by horseradish peroxidase (HRP)-gold¹⁹. Labelling of coated pits and vesicles was commonly observed with this ligand (data not shown).

The distribution of cholera and tetanus toxin surface labelling was quantified by calculating the ratio between the percentage of gold particles bound to each membrane specialization and

Table 1 Association of cholera toxin-coated gold particles with cell surface features

Cell surface feature	% Gold particles associated with the feature	% Surface length occupied by the feature	Ratio
Flat plasma membrane	34.1	55.5	0.6
Microvilli	30.1	34.5	0.9
Smooth microinvaginations	35.8	8.9	4.0
Coated pits	0	1.1	0

The numbers of gold particles found on flat regions of plasma membrane, microvilli, smooth microinvaginations and coated pits on the apical cell surface were counted directly in the electron microscope on sections cut perpendicularly to the substratum. In each case, 35-50 cells (~1.0-1.7 mm of cell surface) were evaluated. Each cell was then photographed and the length of the whole apical cell surface and the length of each surface feature were measured on negatives projected at an appropriate magnification (×46,000 for flat membrane and microvilli; ×92,000 for smooth and coated invaginations) on a graphic tablet connected to an IMSAI microprocessor. The relationship of cholera toxin-coated gold particles to the various features of the cell surface at 2 °C is expressed as the ratio between the percentage of particles associated with a given feature and the percentage of the length of the cell surface occupied by the feature. A ratio of ~1 means that labelling is random. A ratio <1 suggests an exclusion; a ratio >1 indicates a preferential association.

the per cent length of cell surface occupied by that structure. The only preferential binding (with a ratio of 4) of gold-labelled cholera toxin at 2 °C was to the non-coated small membrane invaginations (Table 1). For tetanus toxin, we found a similar but less pronounced initial preferential binding (ratio of 1.5) to non-coated membrane invaginations (see Fig. 3). Furthermore, our qualitative observation that in cells incubated at 2 °C the neck of the flask-shaped membrane invaginations was more frequently labelled than the body region (compare Fig. 1a, b) was confirmed quantitatively (Fig. 3). In view of the rigidity of the membrane at 2 °C, this phenomenon could result from an impaired transfer of the gold tracer into the body of the invaginations. In contrast, after 10 min, and more markedly after 30 min of warming to 37 °C, gold particles were preferentially associated with the body of the smooth microinvaginations; the association of the label with microinvaginations as a whole was also more pronounced than at 2 °C. Cholera toxin surface labelling was reduced by only 1% after 10 min of reincubation to 37 °C, and by 26% after 30 min, indicating a slow rate of internalization. The decrease of surface labelling at 37 °C was more rapid in the case of tetanus toxin (34% after 10 min, 77% after 30 min).

In conclusion, our results show that: (1) at 2 °C toxin-gold complexes bind preferentially to non-coated, flask-shaped microinvaginations; (2) this preferential association becomes more pronounced after warming of the cell to 37 °C and is accompanied by a progressive translocation of the label from the neck to the body of the invaginations; and (3) at 37 °C the label is internalized via small, non-coated vesicles, probably derived from the surface microinvaginations, and accumulates in multivesicular bodies and lysosomes. We cannot, however, exclude the possibility that a small fraction of the label may enter cells through coated pits in the first few minutes after warming to 37 °C, as we did not study time points earlier than 10 min.

Studies on a variety of systems have contributed to the now widely accepted view that receptor-mediated endocytosis proceeds through clathrin-coated pits on the cell surface¹⁻³. These studies concern essentially ligands that bind to membrane receptors thought to be glycoproteins¹⁻³. However glycolipids, and in particular gangliosides, can also serve as selective receptor sites for various macromolecules²⁰⁻²², including cholera⁸⁻¹¹

and tetanus12-15 toxins, but the surface events involved in the internalization of these ligands have received only limited attention²³⁻²⁵. Joseph et al.²⁴ mentioned that cytoplasmic coated vesicles did not participate in the internalization of peroxidaselabelled cholera toxin by neuroblastoma cells. However, their study focused on late events of internalization and the type of surface invagination involved in the early stages of endocytosis was not investigated. The present results demonstrate that both the initial binding and subsequent internalization of goldlabelled cholera and tetanus toxin are mediated by non-coated surface microinvaginations, which suggests that the pathway of endocytosis of a ligand may depend on the chemical nature of its receptor. In this respect, it is interesting that gangliosides, due to their localization in the outer leaflet of the membrane bilayer, may not interact directly with the clathrin coat on the cytoplasmic side of the membrane. The present observations are also consistent with the notion that coated pits may act as molecular filters^{3,26}, concentrating some specific membrane components^{1,6}, but excluding others^{18,26}. The exclusion of gangliosides, in particular, could correlate well with the reported absence of sialic acid groups from coated pits27

In conclusion, our observations together with the previous studies on antibody-induced endocytosis of HLA antigens⁷ strongly suggest that there are at least two distinct pathways

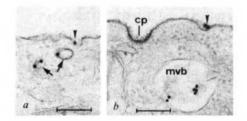


Fig. 2 Internalization of tetanus toxin-gold by cultured liver cells during reincubation at 37 °C for 30 min. Gold particles are present in smooth microinvaginations (arrowheads), in small non-coated cytoplasmic vesicles (arrows) and in a multivesicular body (mvb). There is no labelling of a coated pit (cp). Scale bar, 0.2 μm.

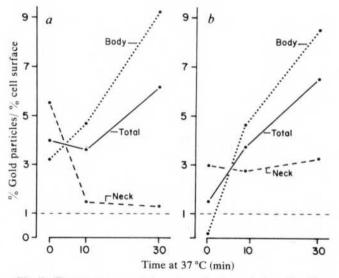


Fig. 3 Temperature- and time-dependent association of gold-labelled cholera (a) and tetanus (b) toxins with non-coated membrane microinvaginations. The time point '0' refers to cells fixed immediately after incubation at 2 °C. The ratio as defined in Table 1 was estimated for the microinvaginations as a whole, and also separately for the neck and body regions. The neck was defined as the region of membrane running from the most constricted part of the invagination up to the flat surface of the plasma membrane, and the body was the portion below the constriction. The neck was found to correspond to 3.4% of the total cell surface length and the body to 5.5%.

for internalization of surface-bound ligands, one involving coated pits and the other involving non-coated microinvaginations.

Received 15 December 1981, accepted 22 February 1982

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We thank Dr D. Brown for revising the manuscript and J. Rial for technical assistance. This work was supported by grant 3.668.80 from the Swiss NSF.

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Conversion of a gonadotropin-releasing hormone antagonist to an agonist

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Gonadotropin-releasing hormone (pyroGlu¹-His²-Trp³-Ser⁴-Tyr5-Gly6-Leu7-Arg8-Pro9-Gly10 amide, GnRH) stimulates pltuitary luteinizing hormone (LH) release. An antagonist, D-pyroGlu¹-D-Phe²-D-Trp³-D-Lys⁶-GnRH (GnRH-Ant), binds to the pituitary GnRH receptor and inhibits GnRHstimulated (10^{-9} M) gonadotropin release from pituitary cultures $(IC_{50} = 2 \times 10^{\times 7} \text{ M})$. GnRH-Ant has no measurable agonist activity at concentrations up to 10^{-6} M. The presence of the D-Lys6 both affords protection against proteolysis and includes an amino group which may be used for derivatization without loss of receptor-binding activity. Formation of the GnRH-Ant dimer by cross-linking of the (lysyl) amino groups of two molecules with ethylene glycol bis(succinimidyl succinate) (EGS) results in a GnRH-Ant dimer joined by a 12-15 Å chain. As the amino terminus on GnRH-Ant is blocked, leaving the D-Lys6 amino group the only reactive group1, the reaction of EGS with GnRH-Ant does not lead to larger polymers. Like the parent compound, this dimer is purely an antagonist. We now show, however, that when antibody (AB) to D-Lys6-GnRH (which cross-reacts with GnRH-Ant) is incubated with excess dimer, a product is formed which consists of a divalent antibody with a GnRH-Ant dimer attached to each arm: AB-((GnRH-Ant)-EGS-(GnRH-Ant))2. In contrast to the parent compounds, this conjugate is an agonist, stimulating LH release from pituitary cultures. Our results suggest that a pure antagonist becomes an agonist when it is capable of bringing two molecules within critical distance, (15 Å < d < 150 Å). The data indicate that formation of the receptor microaggregate itself is sufficient to stimulate a transmembrane response system.

Figure 1 shows the ability of GnRH-Ant to inhibit GnRHstimulated LH release from pituitary cell cultures. GnRH-Ant was an effective inhibitor of stimulated LH release; alone it has no detectable agonist action at concentrations up to 10⁻⁶ M. Figure 2 shows the elution pattern (G-25 column chromatography) of (1) authentic 125 I-GnRH-Ant, (2) EGS+GnRH-Ant dimerization reaction mix and (3) the EGS+GnRH-Ant dimerization mix in which the cross-linking volume was 10 times greater than in (2). Although complete separation of the monomer and dimer was not possible, the leading edge (see bracket in Fig. 2) consisted of fractions of GnRH-Ant dimer which contained <0.5% monomer. To provide evidence for the formation of a dimer, EGS cross-linking was performed in a 10-fold greater than normal incubation volume (a condition which would not favour the two second-order reactions required for the formation of the GnRH-Ant dimer). This resulted in an elution pattern in which a smaller percentage of the total GnRH-Ant was cross-linked by EGS.

We next analysed the activity of AB-((GnRH-Ant)-EGS-(GnRH-Ant))₂. In this conjugate, one molecule of each GnRH-Ant dimer is attached to each antigenic binding site and is therefore unavailable to the receptor. The other molecule of each dimer is available to the receptor, although it is bound to

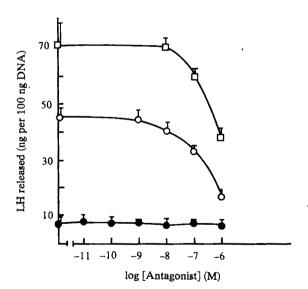


Fig. 1 Antagonist activity of GnRH-Ant in the cell culture bloassay: 2-day pituitary cell cultures² were incubated with 10^{-8} M (\Box) , 10^{-9} M (\bigcirc) , or no (\bullet) GnRH at the indicated concentration of antagonist. After 5 h incubation, the medium was assayed for LH by radioimmunoassay (RIA) using rat anti-LH antisera (LHS-4), highly purified rat LH for labelling (LH-I-4) and rat LH standard (LH-RP-1), which were made available by a grant from the NIAMDD Hormone Distribution Office (prepared by Dr A. Parlow). The assay was performed as previously reported², except that a second antibody was used to separate the bound and free ¹²⁵I-LH. The intra-assay variance was 7% and the inter-assay variance 12%. As indicated, LH release was normalized for slight differences in cell numbers in different wells by DNA determination 15.

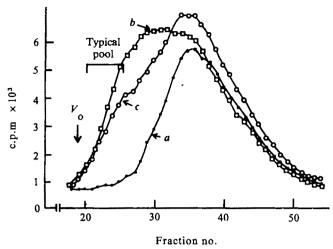


Fig. 2 Elution profiles of 125-I-GnRH-Ant (a), the EGS-GnRH-Ant-containing dimerization reaction mixture (showing a typical pool of the purified dimer (b) and the identical reaction conducted in a 10-fold greater volume (c). GnRH-Ant (prepared by J.M.S., batch LR307/29-39) was iodinated using chloramine T I and unlabelled hormone were removed from the GnRH-Ant by CM-cellulose column chromatography, as described previously except that the labelled analogue was eluted from the column with 200 mM sodium phosphate, pH 4.5. Maximum binding (assessed by binding with excess GnRH antiserum (no. 9113), prepared and used as in ref. 7) ranged from 89 to 92% in different preparations and specific activity (assessed by self-displacement assay with pituitary plasma membrane fragments 17) was $\sim 1~\text{mCi}~\mu\text{g}^{-1}$. The product was stored in the elution buffer at 4 °C for up to 2 weeks. At that time, >90% of the hormone (in different preparations) was intact as judged by electrophoresis¹⁷. Ten nmol of GnRH-Ant in . Ten nmol of GnRH-Ant in 10 μ l of 10 mM sodium phosphate, pH 8.5, and 20 μ l (~0.05–0.15 μ Ci) of ¹²⁵I-GnRH-Ant in 200 mM sodium phosphate, pH 8.7, were combined with 5 nmol of EGS (Pierce Chemical, Illinois) in 1.5 µl of dimethyl sulphoxide (DMSO, redistilled from Fisher). The EGS was stored in the dark at -70 °C and was dissolved in DMSO immediately before use. As EGS has 2 mol of reactive groups per mol, the reactive groups were present in equimolar concentration with the lysyl e-amino in GnRH-Ant. After 1 h at 18 °C, the reaction was terminated by dilution with 270 µl 40% ethanol, 10 mM sodium phosphate, pH 8.5, and immediately applied to a G-25 column (10×260 mm) previously equilibrated in the ethanolic buffer. Positions of cluting substances were determined by radioactivity and confirmed by RIA (using antibody 9113 at final titre of 1:7,000, radioiodinated GnRH and authentic GnRH-Ant standard).

the antibody via the 15 Å chain and the other molecule of GnRH-Ant. The product thus consists of two molecules of GnRH-Ant available to the receptor although separated from each other by ~150 Å. Figure 3 shows the ability of column fractions containing AB-((GnRH-Ant)-EGS-(GnRH-Ant))2 (the 'conjugate' which elutes in the V_0) to stimulate LH release from pituitary cultures. In contrast to GnRH-Ant alone, the conjugate displayed agonist action, evoking release of 9 ± 1.0 ng LH per 20 µl of medium compared with 0.8±0.1 ng LH per 20 μ l medium for basal release and 13.6 \pm 0.9 ng LH per 20 μ l of medium released in response to 10⁻⁷ M GnRH during a 5 h period. Stimulated release was blocked by 2 mM EGTA (in the presence of 'normal' 1.25 mM extracellular Ca2+) and by 5 µM Pimozide (which behaves as a calmodulin antagonist in this system²). In addition, neither the antibody alone (Fig. 3) nor the GnRH-Ant dimer alone stimulated measurable release (0.9± 0.1 ng LH per 20 µl medium). The reduced pepsin AB fragment (monovalent AB) conjugated with GnRH-Ant dimer did not stimulate release, indicating the requirement for bivalency to produce this effect.

GnRH occupancy of its receptor stimulates pituitary gonadotropin release by a calcium-mediated mechanism³ involving calmodulin^{2,4}. Occupancy leads to patching, capping and internalization⁵, although neither internalization of the releasing hormone nor large-scale patching and capping of the hormone-receptor complex are required for stimulation of LH release^{6,7}. The results reported here support the view that formation of a microaggregate of the GnRH receptor is sufficient to transduce the signal across the plasma membrane and to elicit cell responses if the separation distance between ligands is increased to a critical point. Thus, although both agonists and antagonists may bind to similar sites on the receptor, the latter is unable to produce the active functional unit. The GnRH-Ant dimer possesses only a 15 Å separation which appears to be inadequate to bridge receptor binding sites; so it cannot stimulate release. The added length due to the antibody indicates that the 'correct' bridge length is ~150 Å.

It is unlikely that the releasing hormone itself bridges two receptors because it is neither multivalent nor sufficiently long. A more likely possibility is that receptor occupancy by an agonist alters the receptor structure so as to increase the probability of microaggregation. For example, occupancy (by an agonist) might increase mobility of the receptor in the plasma

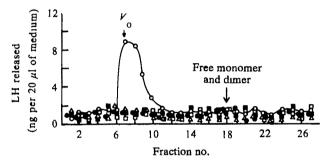


Fig. 3 Effect of column fractions containing AB-dimer conjugate on LH release from pituitary cell cultures. 120-160 ng (GnRH-Ant)2-EGS collected from the leading edge of the G-25 ∞lumn described for Fig. 2 (which contained <0.5% of the free GnRH-Ant, or EGS-GnRH-Ant monomer complex, estimated from other column eluant patterns) were lyophilized in a glass tube. Rabbit anti-D-Lys⁶-GnRH (antiserum no. 5111 prepared by immunization with D-Lys⁶ coupled to bovine serum albumin (BSA) with glutaraldehyde) was added without additional purification in an amount sufficient to bind 8-10% of the dimer. The AB was added in a final volume of 50 µl (with Medium 199 containing 0.3% BSA and 2 mg NaN3). The reaction was carried out for 10 h at room temperature and then for 2 days at 10 °C. Immediately before the cell bioassay, the solution was applied to a 12 ml calibrated G-75 column (10×260 mm) equilibrated in Medium 199/BSA. Twenty drop fractions (~300 µl) were collected and brought to 1.0 ml with Medium 199/BSA. The conjugate elutes in the V_0 . Where monovalent antibody is specified, the reduced 18 pepsin fragment 19 of antibody 5111 was substituted for intact antibody in the conjugation described above. In this case ammonium sulphate-fractionated IgG was dissolved in 50 mM acetate buffer, pH 4.5, and digested with pepsin (Worthington Biochemical) at an enzyme/substrate ratio of 1:100 at 70 °C for 50 min. Digestion was stopped by neutralization with 1 M Tris-HCl, pH 8.0 and 0.1 M NaOH. Precipitated material was removed by centrifugation and 2-mercaptoethanol was added to the supernatant at a final concentration of 0.75 mM. The solution was maintained at room temperature for 1 h, then cooled in ice-cold water. An equal volume of 0.75 mM iodoacetamide (previously degassed and cooled at 4 °C) was added and triethanolamine added to maintain the pH at 8.0 ± 0.2 . After 1 h the solution was dialysed against three (2 h each) 100 vol changes of 0.9% NaCl then lyophilized. This product was >95% cleaved as judged by the inability to precipitate ¹²⁵I-D-Lys⁶-GnRH with protein A. Cell cultures (described in Fig. 1 legend) were incubated with the aliquots from the column fractions in a final volume of 1.0 ml of Medium 199 containing 0.3% BSA. The conjugate $(n = 7, \bigcirc)$ stimulates LH release (by RIA as described for Fig. 1) which is blocked in the presence of 10 μ M Pimozide ($n = 3, \triangle$), or 2 mM EGTA $(n=3, \blacksquare)$. A conjugate prepared with monovalent antibody $(n=3, \Box)$ or bivalent antibody alone $(n=3, \bullet)$ did not stimulate release. Standard errors of the means, which are not shown for clarity, were <15% of the indicated value.

membrane by releasing it from anchoring; alternatively, a conformational change might occur such that complementary binding sites are exposed. It is also possible that occupancy activates enzymes which catalyse the formation of disulphide8 or peptide9 bonds and thereby link receptors. An antagonist would be viewed as occupying the binding site without effecting these changes.

Microaggregation of plasma membrane receptors seems to be required for the activation of some other plasma membraneregulated systems. In the case of epidermal growth factor (EGF)-stimulated mitogenesis in 3T3 fibroblasts 10, , cyanogen bromide treatment of EGF results in a molecule (CN-EGF) which binds to the EGF receptor but does not elicit mitogenesis. Addition of bivalent EGF antibody, which presumably brings together the occupied receptors, results in activation of mitogenesis. Further, anti-insulin receptor antibodies can stimulate insulin-like functions in target tissues¹¹. Bivalent antibodies are required for this stimulation¹², indicating the importance of a receptor microaggregation in this process also.

Divalent antigens such as EGS-(GnRH-Ant)₂ have been useful in the determination of the bridge length between the binding sites on antibodies¹³ and in the demonstration of the bivalency of IgG molecules¹⁴. The technique described here provides a general means of preparing a bivalent antibody that binds to the active site of a receptor from an antibody which was initially prepared by immunization with the ligand.

The work was supported by the Mellon Foundation through the Ford-Rockefeller Mellon Program for Targeted Research in Reproduction and by NIH grant HD 13220. P.M.C. is the recipient of grant RCDA HD 00337 and a senior fellow in the Center for the Study of Aging and Human Development. We thank Drs J. Blum, N. Kirshner, S. Pizzo and T. Slotkin for commenting on the manuscript, and Dr M. Karten of the Contraceptive Development Branch (NIH) for helping to locate the antagonist used in this study.

Received 17 November 1981, accepted 5 March 1982

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Axon segments sprout at both ends: tracking growth with fluorescent D-peptides

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In the nervous systems of many animals, including vertebrates, isolated axon segments can survive for weeks or even months 1they can participate in regeneration^{4,5} and in some cases receive synapses from regenerating axons^{6,7}. Axon segments in cell culture exhibit limited growth⁸⁻¹⁰. Although lengths of axon may grow in more intact systems¹¹⁻¹⁴, direct evidence for this has been difficult to obtain, partly because of the difficulty of marking isolated axon segments. We have injected fluorescently labelled synthetic D-peptides¹⁸ into identified neurones in the leech central nervous system to mark axon segments. We report here that individual segments can initiate growth at both ends and grow for days, both in organ culture and in vivo. These results support the hypothesis that axon growth is not essentially polar and suggest a novel mechanism by which axon segments may assist nerve regeneration.

Cell bodies of pressure (P) sensory neurones¹⁶ were injected with either rhodamine- or fluorescein-conjugated D-peptide15 which spread throughout the cell (see Fig. 1 legend). The synthetic peptide consists of 12 amino acids in the D-configuration, making it resistant to proteolytic enzymes¹⁵. Two days after injection into cells within cultured chains of ganglia, axon segments 2-4 mm long were produced by crushing or cutting the nerve cord at two sites between adjacent ganglia (Fig. 1). Growth was traced at 1- or 2-day intervals by viewing the preparation with an image-intensified camera and epifluor-escent illumination¹⁷ (Fig. 2). At the end of the experiment, preparations were fixed and viewed by conventional fluorescence microscopy (Fig. 3).

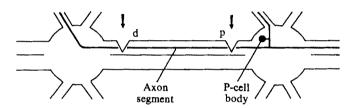


Fig. 1 A schematic diagram of the nerve cord preparation. P-cell bodies were pressure-injected using glass microelectrodes filled with either rhodamine-D-peptide (2% in 0.2 M KCl with 0.2% Fast Green FCF) or fluorescein-D-peptide (2% in 0.025 M Trischloride buffer pH 11, with 0.2% Fast Green FCF and 0.1 M KCl). Injections were performed in chains of ganglia pinned to silicone rubber (Sylgard 184, Dow Corning) and maintained in supplemented Leibowitz-15 culture medium 19,30. After 2 days, the nerve cord was cut or crushed in two places (arrows) between adjacent ganglia, creating an axon segment with proximal (p) and distal (d)

Axon segments began sprouting within the first day after cutting and continued to grow for at least 6 days (Fig. 2). After longer periods, increased background autofluorescence at the lesion site made it difficult to detect fine branches (or neurites). Typically, fine sprouts emerged from the ends of the severed axon, and branched and grew in several directions within the nerve cord, including backwards along the axon (Fig. 3). Some neurites had at their tips a swelling which seemed to be a growth cone, and small swellings or varicosities were seen at points along the sprouts, features characteristic of growing axons in diverse systems^{9,18}. Of 84 axon segments examined, 42 grew at both ends, 33 grew only at the distal end, one grew only at the proximal end and 8 did not grow detectably. The distal end usually sprouted more profusely than the proximal end. Although individual branches occasionally grew 100 µm per day for up to 2 days, their average growth rate over a 6-day period was 35 µm per day or less, a rate comparable to that seen for isolated leech neurones in culture19. There was no obvious difference in the amount of growth after cuts or crushes, although following cuts those neurites that extended forwards appeared to grow across the cut surface of the nerve cord.

The two cuts or crushes separated axons into three pieces: an axon still attached to the cell body, the axon segment and the piece distal to the axon segment (Fig. 1). All the cut ends sprouted and grew at comparable rates.

To determine whether axon segments can also grow in vivo, P-cells were injected in situ²⁰ with fluorescent D-peptide. One or two days later, axons were severed by crushing the nerve cord (Fig. 1) and after a further 6 days, chains of ganglia were

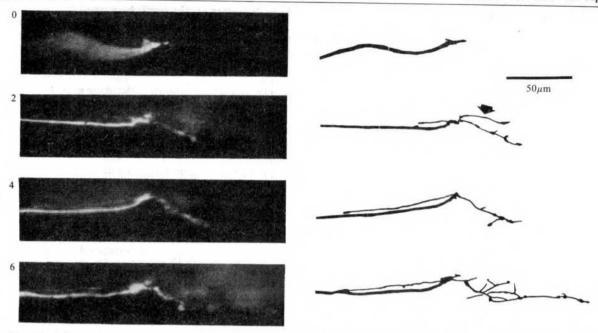


Fig. 2 Growth of the proximal end of an axon segment. Panels on the left are photographs of the video monitor screen at 2-day intervals (number of days indicated at the left) beginning 30 min after the axon was severed by crushing the nerve cord. The shallow depth of field of the objective lens reduced the amount of arborization visible in any single photograph of the monitor screen. Therefore, for each time point, tracings were made from the screen while focussing through the preparation (right-hand panels). The arrowhead indicates a neurite that apparently retracted between days 2 and 4. The preparation was viewed by epifluorescent illumination through a ×40 water immersion objective and suitable filters (Zeiss), in combination with a silicon intensified target (SIT) camera (RCA TC 1040/H), video monitor (RCA TC 1209) and video recorder (Panasonic, NV 8030). To determine the diameter of branches that were detectable with the video system, 2% HRP (Sigma, Type VI) in 0.2 M KCl with 0.2% Fast Green FCF was injected before the fluorescent D-peptide. The tissue was fixed, reacted with diaminobenzidine, and prepared for electron microscopy²². Electron microscopic examination of HRP-filled branches that had been viewed by fluorescence in the live preparation showed that branches of diameter 0.3 μm or less could be seen with the SIT camera.

removed, fixed and examined by fluorescence microscopy. Of 11 segments examined, 5 grew at both ends, 3 grew only at the distal end, one grew only at the proximal end and 2 did not grow detectably. Because the axon segments contained D-peptide, an effect of the peptide injection on growth must be considered. The fluorescent D-peptides we have used do not affect development when injected into single embryonic cells15, but we found that axons attached to P-cell bodies injected with Dpeptide sprouted less than uninjected controls (our unpublished results). This suggests that the growth of axon segments observed here may underestimate the normal growth. Axon segments of P-cells injected with horseradish peroxidase (HRP) sprouted less frequently and profusely than those injected with Dpeptide, suggesting that injected HRP inhibits nerve growth. In this context it is interesting that HRP damages frog motor nerve terminals21

The varicosities along neurites of axon segments resemble sites of sensory cell synapses in the leech^{22,23}. Synapses are formed by colchicine-treated segments of frog motor axons during their brief period of sprouting¹⁴, and leech axon segments, which survive for longer periods, may also form synapses.

The present study of leech sensory cells shows that isolated axon segments can initiate growth at both ends and grow for at least 6 days. These results support the idea that growth can be controlled locally within the cell^{24,25}. They also demonstrate that although the axonal cytoskeleton is organized in a polar fashion²⁶ and some²⁷ but not all²⁸ axonal transport is directional, axonal growth need not be intrinsically polar. Because the distal stump can be important in accurate regeneration, the possibility must be considered that sprouting by the proximal end of the stump facilitates contact and recognition between the regenerating axon and its severed stump. The source of materials necessary for growth by axon segments is unknown; they could originate either from within the axon or be transported to it from surrounding glial cells²⁹. Although the mechanism by which axons sprout and elongate is little understood, the present experiments add to the growing body of evidence indicating that

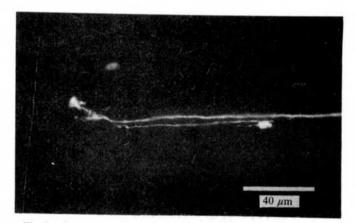


Fig. 3 A montage of fluorescence micrographs of the distal end of an axon segment filled with fluorescein—D-peptide and fixed 4 days after the axon was severed by cutting the nerve cord. The preparation was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 and mounted in glycerol at pH 9.5. Several branches extended forwards and a single neurite, tipped with a large swelling resembling a growth cone, grew backwards along the axon segment.

the axon can have significant functional independence from the cell body.

We thank David Weisblat for supplying the D-peptides used in this study, Barbara Thomas for technical assistance, and Ellen Elliott and Sam Ward for useful comments on the manuscript. This work was supported in part by a NATO postdoctoral fellowship to A.M., a McKnight Neuroscience Development Award to K.J.M. and USPHS grant NS15014.

Received 22 December 1981; accepted 23 February 1982.

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Gating currents associated with potassium channel activation

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The voltage dependence of channel-mediated ion conduction in excitable membranes is thought to be due to rearrangement of charged groups or dipoles within the membrane in response to changes in transmembrane potential¹. This charge movement, or gating current (I_s) , has been detected in squid giant axons for Na+ channel activation^{2,3}, but no I_s has been detected which has kinetics similar to those of K^+ channel activation. This is probably due to the relatively slow time course of K+ channel activation at the temperatures normally used (6-8 °C). We report here that when the temperature is raised to 20-22 °C to increase the rate of channel activation, a new component of I_{s} is detected which shows kinetics and voltage dependence similar to those of K+ channel activation at the same temperature. In addition, the net charge moved is consistent with estimates of K channel density. We suggest that this component of I_2 is due to the charge movement associated with the rate-limiting step of K⁺ channel activation.

Experiments were performed on internally perfused squid giant axons at 20-22 °C using standard voltage-clamp techniques⁴. The internal solution contained either a mixture of potassium fluoride and potassium glutamate (for K⁺ currents) or N-methylglucamine(NMG) fluoride and NMG-glutamate (for gating currents). The external solution consisted of Na⁺-free Tris-seawater with tetrodotoxin (TTX) added. The holding potential, -60 mV, was chosen to inactivate partially the Na channel gating current5.

Figure 1 shows K⁺ ionic and gating currents recorded at 21 °C from the same axon after a voltage step to three different potentials. In all three cases the gating current contains two clearly defined components: the fast component, which is the gating current associated with Na+ channel activation, and the slow component, which is undetectable at 6 °C, and is too slow to be associated with Na+ channel activation but has a time course similar to the K⁺ currents. Figure 2a shows the voltage dependence of the time constants for K⁺ channel activation (τ_K) and the slow phase of $I_{\rm g}(\tau_{\rm g})$ for potentials from $-30~{\rm mV}$ to $+30~{\rm mV}$. The two sets of time constants correlate fairly well, indicating that this slow component may be related to K+ channel activation. Dibucaine was included in the external solution in the gating current measurements to block some of the Na+ channel gating current. At the concentration used in this study (0.2 mM), dibucaine blocks $\sim 50\%$ of the Na⁺ channel I_g without significantly affecting either the K+ currents or the slow component.

To estimate the amount of charge (Qs) responsible for the slow $I_{\rm e}$, the gating current traces were integrated from the break point in the relaxation to the steady-state level for both ON and OFF gating currents. Although this procedure minimizes any contribution from the fast component, it also ignores any slow charge movement that may have occurred from the start of the voltage step to the start of the integration period. However, as nothing is known about the shape of the early part of the slow I_z , we consider this an adequate method of estimation. We can assign an upper limit to the amount of charge movement that would not be accounted for by the above method by assuming that the slow phase extrapolates exponentially to the beginning of the pulse. When the amount of charge moved is determined in this way, we find that only 10-20% of the charge moved is missed by the original method. Figure 2b shows the voltage dependence of $Q_{s,ON}$ measured by this method: the charge moved is zero at voltages more negative than -80 mV, rises sharply as the voltage becomes more positive and finally saturates at voltages greater than zero. The saturation value of $Q_{s,ON}$ was 508 electronic charges per μm^2 ; the value for all experiments was 500 ± 90 electronic charges per μm^2 ($\pm s.d.$ of 13 determinations). $Q_{s,OFF}$ shows a similar voltage dependence and saturation value. The ratio, $Q_{s,ON}/Q_{s,OFF}$, was found to be 1.1 ± 0.2 (±s.d. of 10 determinations), as expected for a true capacitative current.

Figure 2b also shows the voltage dependence of g_K , the K^+ chord conductance (corrected for the non-linearity of the instantaneous current-voltage relation), which is a measure of the fraction of K+ channels that are in the open state. The voltage dependence of g_K is similar to that of the charge move-

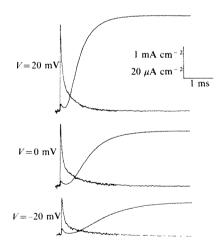


Fig. 1 Potassium $(I_K, \text{ smooth traces})$ and gating $(I_g \text{ noisy traces})$ currents recorded at 21 °C in the same axon in response to depolarizations from -60 mV to three different voltages. Leakage and capacitative currents were subtracted using the P/4 technique with the subtracting holding potential at -110 mV for both currents. I_{K} records are single sweeps and show gating current at early times. I_a traces are the average of 20 records. The external solution contained 440 mM Tris-HCl, 50 mM CaCl₂, 0.3 µM TTX pH 7.2. For gating current measurements, dibucaine (0.2 mM) was added to the external solution. Internal solutions were either 66 mM KF, 133 mM K-glutamate, 440 mM sucrose, 10 mM Tris-HCl pH 7.2 (for I_K) or 133 mM NMG-glutamate, 66 mM NMG fluoride, 440 mM sucrose, 10 mM Tris-HCl pH 7.2 (for Ig). Axon JUL211B.

ment, except that the conductance curve is shifted to the right along the voltage axis. Similar behaviour has been observed for the Na+ channel and is considered evidence for the existence of multiple closed states in voltage-dependent equilibria6.

The agreement between the kinetics and voltage dependence of the slow I_g and K^+ channel activation may, however, be fortuitous. K^+ channel activation and Na^+ channel inactivation have similar kinetics and voltage dependence, thus a linkage between Na⁺ channel inactivation and the slow I_* will also seem to hold for K⁺ channel activation. To test this possibility, we examined the effect of an 8-ms prepulse to -110 mV on the K+, Na⁺ and gating currents in response to a voltage step to 0 mV. The K⁺ currents showed a well-defined Cole-Moore shift⁷ with the activation of channels being delayed markedly (~200 µs) when the prepulse was applied (Fig. 3a). The same delay was seen for the slow I_g (Fig. 3b). Na⁺ channel inactivation, on the other hand, showed no detectable Cole–Moore shift (Fig. 3c); if it had, then the Na+ current peak after the prepulse would have shown a definite broadening, but this was not the case.

The results presented here strongly suggest that the slow I_{g} is caused by the charge movement associated with the ratelimiting step of K+ channel activation. The time course of both K^+ channel activation and the slow I_a are similar at all voltages examined. In addition, the amount of charge responsible for the slow I_g is consistent with the amount of charge moved as a K⁴ channel opens. Using the saturation value of the Q_s-V relationship (~500 charges per µm²) and estimates of the K⁺ channel density in squid giant axons (70 per μ m²; refs 8, 9), we

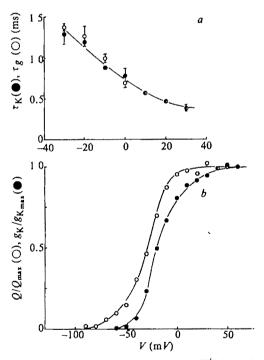


Fig. 2 Correlation of charge movement with K+ channel activation. Conditions were as described in Fig. 1 legend. a, Time constants of K^+ activation, τ_K (\bullet), were determined by fitting the current relaxation after the initial delay to a single exponential. The time constants of the slow $I_{\mathbf{g}}$, $\tau_{\mathbf{g}}$ (O), were determined by fitting the decay of the slow I_g to a single exponential. Each value is the mean ±s.d. of single determinations from three different axons: JUL211B, JUL221A and JUL221B. b, The charge moved during the slow component of ON gating current was determined as described in the text and normalized to the maximum value (508 electronic charges per μm^2 in this case) and plotted as a function of voltage (O). Also shown is the voltage dependence of the K+ chord conductance corrected for the non-linearity of the instantaneous current-voltage relation and normalized to its maximum value (●). The holding potential was -60 mV and the subtracting holding potential, -120 mV. In all cases the test pulse was preceded by an 8-ms prepulse to -110 mV to make the break point more obvious (see Fig. 3). Axons AUG181A and AUG221A.

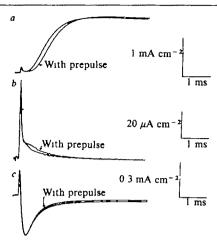


Fig. 3 Effect of a hyperpolarizing prepulse on $K^+(a)$, gating (b), and Na⁺(c) currents in response to a voltage step to 0 mV. K gating currents were measured as described in Fig. 1 legend. Trace c, which shows Na+ currents preceded by gating current, was obtained in an external solution containing 90 mM NaCl, 350 mM Tris-HCl, 50 mM CaCl₂ pH 7.2; the internal solution was the NMG solution used for gating current measurements. Where indicated, the depolarizing step was preceded by an 8-ms prepulse to $-110 \,\mathrm{mV}$. The current trace in c, obtained without a prepulse, was multiplied by 1.15 to correct for the difference in peak Na+ and gating currents. This scaling was done to account for the removal of some resting inactivation by the prepulse. Temperature, 21 °C. Axons JUL221B (I_K and I_g) and AUG261A (I_{Na}).

obtain a value of ~7 charges per channel. This agrees well with the 5-6 charges per channel expected from macroscopic conductance measurements 10,11 It is possible, however, that not all the slow charge movement detected is due to K+ channel gating currents. There may be contributions from other sources (probably Na+ channel inactivation), thus the values of Q. reported here are upper limits. Finally, the slow I_s shows a Cole-Moore shift identical to that seen for K^+ channel activation. This property represents the strongest argument we have that this slow $I_{\rm g}$ is not a charge movement associated with Na⁺ channel inactivation, but actually is the K+ channel gating current.

Gilly and Armstrong¹² recorded a slow component of I_g at 8°C that correlated with the existence of functional K+ channels. This charge movement was faster than the development of K⁺ currents, which led the authors to suggest that it represented the charge movement associated with a fast step in the activation process. The component described here, however, is not removed by destruction of K+ channels due to removal of K+ ions and, furthermore, has kinetics identical to K+ channel activation. We suggest that the slow I_s described here represents the charge movement associated with the rate-limiting step of K+ channel activation, while that described by Gilly and Armstrong is associated with a later (the last?) step in the activation process. Such a scheme has been proposed¹³ to explain the bursting patterns seen in single-channel recordings of K+ channels. Studies of the kinetic behaviour of both these charge movements in response to different pulse protocols should prove valuable in the construction and testing of models for K⁺ channel activation, as gating currents can provide information about transitions between closed states that cannot be obtained from conductance measurements

This work was supported by USPHS grants GM 30376 and 1-T32-NS 07101.

Received 30 November 1981; accepted 4 March 1982.

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Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide

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The C-terminal α -amide structure is a characteristic feature of many biologically active peptides^{1,2}. Using a novel chemical method for the detection of peptide amides3, we have isolated two naturally occurring peptides, peptide HI (PHI) and peptide YY (PYY), from extracts of porcine intestine and have shown that these peptide amides represent previously unknown biologically active peptides4. PHI is structurally and biologically similar to vasoactive intestinal peptide (VIP) while PYY shows similarities to pancreatic polypeptide (PP)6. Preliminary studies indicated that PHI and PYY may both be present in brain as well as in intestine4. We report here the isolation from brain extracts of a peptide amide that was thought to be PYY. However, we found that the peptide, while having distinct structural and biological similarities to both PYY and PP, is a previously uncharacterized peptide, which we designate neuropeptide Y (NPY).

NPY-containing fractions were detected from the amounts of tyrosine amide released after treatment of the lyophilized fractions with trypsin or thermolysin using a method described elsewhere^{3,5}. The brain extract and its methanol-soluble peptide fraction were prepared using procedures similar to those described previously⁷; details are given in Fig. 1 legend. The methanol-soluble peptide fraction (1.8 g), obtained from 400 kg of porcine brain, which was also used for the isolation of VIP8, was first subjected to gel filtration on a Sephadex G-25 column. The NPY fractions, which also contain secretin and VIP, were pooled and lyophilized and the lyophilized material (690 mg) was chromatographed on CM-cellulose (Fig. 1). The peptide was eluted with 0.02 M ammonium bicarbonate containing 0.025% mercaptoethanol; this procedure yielded a total of 76 mg of NPY fractions which were free from secretin and VIP. These fractions were further purified by HPLC on a reverse-phase silica gel column, μ Bondapak C-18 (Waters); see Fig. 2. The peptide was eluted with 40% ethanol/5 mM ammonium acetate/0.2% acetic acid in isocratic conditions. This step yielded a total of 16 mg of highly purified NPY fractions. A pure peptide preparation was obtained from these fractions by re-chromatography on a HPLC column using a linear gradient elution system consisting of 0.12% trifluoroacetic acid/water and 0.1% trifluoroacetic acid/acetonitrile (Fig. 3)

The peptide preparation was found to be homogeneous by amino acid analysis, N- and C-terminal determinations, and by HPLC and TLC analyses. HPLC showed that the elution profiles of this peptide were different from those of either PYY or PP. Amino acid analysis revealed that the peptide consists of 36 amino acid residues (Table 1). This peptide has tyrosine as its N-terminal residue, tyrosine amide as its C-terminal and five tyrosine (Y) residues per molecule. It was therefore designated neuropeptide Y (NPY).

The intestinal peptide PYY, like neuropeptide Y, is 36 residues long and contains an N-terminal tyrosine and a C-terminal tyrosine amide^{4,6}. PP is also 36 amino acid residues

Table 1 Amino acid compositions and N- and C-termini of the porcine peptides NPY, PYY and PP

Amino acid	NPY	o. of residues PYY	PP
Ala	4.1 (4)	4	5
Arg	4.0 (4)	4	4
Asx	5.0(5)	2	3
Glx	3.0 (3)	5	5
Gly	1.1 (1)	1	1
His	1.0 (1)	1	0
Ile	2.0(2)	0	1
Leu	3.0 (3)	4	3
Lys	1.0(1)	1	0
Met	0.0 (0)	0	2
Pro	3.9 (4)	4	2 5
Ser	2.0(2)	3	0
Thr	1.0 (1)	1	2
Tyr	5.0 (5)	5	4
Val	0.0 (0)	1	1
Total no. of residues	36	36	36
N-terminus	Туг	Tyr	Ala
C-terminus	Tyr-NH ₂	Tyr-NH ₂	Tyr-NH ₂

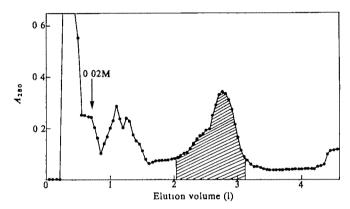


Fig. 1 Ion-exchange chromatography of NPY fraction on CMcellulose. Porcine brains (400 kg) without cerebellum and pituitary were boiled in water for 10 min, frozen and then extracted with 0.5 M acetic acid (1,000 l) for 24 h. Following filtration, peptides in the filtrate were adsorbed on alginic acid pH 2.7, eluted with 0.2 M HCl and precipitated with NaCl at saturation. The wet weight of peptide precipitate collected was ~800 g. This crude peptide material was dissolved in 161 of water then 2 vol of ethanol were added, and the pH adjusted to 7.2. After filtration, 3 vol of water were added to the filtrate, peptides in the solution were adsorbed on to alginic acid, eluted, precipitated and collected as described above. This peptide precipitate (80 g wet weight) was dissolved in water and then re-precipitated with NaCl, pH 4.0. The peptide material was suspended in methanol (41) containing 0.1% mercaptoethanol and extracted at room temperature for 15 min. After filtration, the methanol extract was neutralized by adding methanolic NaOH solution. The precipitate formed after neutralization was removed by filtration and the pH of the methanol solution readjusted to 2.7 (glass electrode) by addition of methanolic HCl. Peptides in the methanol solution were precipitated by addition of 3 vol of ether. The precipitate was collected by suction filtration and dried under reduced pressure—this yielded 1.8 g of the methanol-soluble peptide fraction. This fraction was subjected to gel filtration on a Sephadex G-25 column in 0.2 M acetic acid and the NPY-containing fractions were pooled and lyophilized. The lyophilized fraction (690 mg) was applied to a CM-cellulose column (5×20 cm) equilibrated with 0.01 M ammonium bicarbonate containing 0.025% mercaptoethanol and initially eluted with the same buffer. NPY was recovered after subsequent elution with 0.02 M ammonium bicarbonate containing 0.025% mercaptoethanol. After lyophilization, an aliquot of each fraction was subjected to the chemical assay. The NPY fractions that emerge at 2,050-3,100 ml (indicated by shading) weighed a total of 76 mg after lyophilization.

long and known to have a C-terminal tyrosine amide9. However, the amino acid composition of NPY differs from those of PYY and PP (Table 1), but it does have structural similarities to PYY and PP. PYY and NPY, in addition to identical N- and Cterminal amino acids, have equal numbers of Ala, Arg, Gly, His, Lys, Pro, Thr and Tyr residues. Further evidence of sequence similarities was provided by analysis of the tryptic fragments. Treatment of PYY with trypsin yielded five fragments⁶; trypsin treatment of NPY also yielded five fragments. two of which seemed identical to the corresponding fragments of PYY. Moreover, the N-terminal amino acids of all five tryptic fragments of NPY determined by the dansyl chloride method¹⁰ were identical to those of the corresponding fragments of PYY. Furthermore, preliminary amino acid sequence studies of the NPY tryptic fragments using the dansyl-Edman procedure¹¹ strongly suggest that NPY has further sequence similarities to PYY and PP. We have previously reported that PYY and PP are structurally related and together form a new peptide family⁶: NPY may be the third member of this novel peptide family.

PYY and NPY seem to have similarities in biological activities as well as in structure. NPY inhibited secretin-stimulated pan-

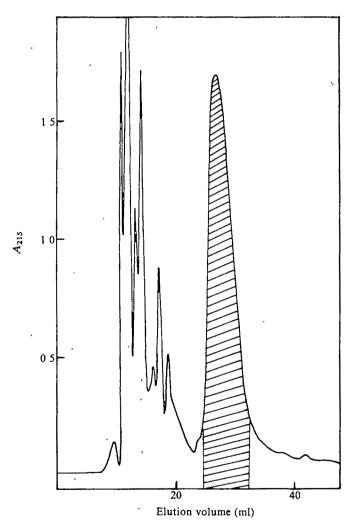


Fig. 2 A typical HPLC elution profile of the NPY fractions (see Fig. 1). The NPY fractions (2 mg per run) were applied to a reverse-phase HPLC column (µ. Bondapak C-18, 7.8×300 mm) and eluted with 40% ethanol/5 mM ammonium acetate/0.2% acetic acid at a flow rate of 2 ml min⁻¹ in isocratic conditions. The HPLC instrument (Waters) consisted of an injector (U6K), two pumps (M-6000 A), a UV detector (450 variable) and a solvent programmer (Model 660). The peak fractions were collected and concentrated to one-fifth of the original volumes under reduced pressure. After the original volumes were restored by adding water, the fractions were lyophilized and an aliquot of each was subjected to the chemical assay The NPY peak is shaded.

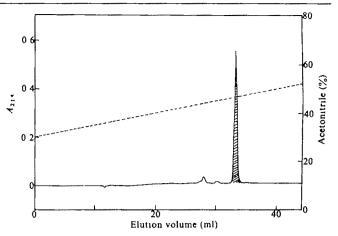


Fig. 3 HPLC elution profile of the NPY peak (see Fig. 2). The NPY fraction (25 µg) was re-chromatographed on a HPLC column (μ Bondapak C-18, 7.8×300 mm) using a linear gradient elution system (solvent A, 0.12% trifluoroacetic acid/water; solvent B, 0.1% trifluoroacetic acid/acetonitrile) at a flow rate of 2 ml min⁻¹ The NPY fraction (shaded area) was lyophilized as described in Fig. 2 legend, and then subjected to amino acid analysis and Nand C-terminal determinations.

creatic secretion in the anaesthetized cat (to be published) but was less potent than PYY⁶. PP is also known to inhibit pancreatic secretion in the dog^{12,13} but has no such effect in the cat.

Recently, many gut peptides originally isolated from the gastrointestinal tract have also been found in the central nervous system^{14,15}. However, despite increasing immunochemical evidence for the existence of many such gut-brain peptides, only a few have been isolated from both tissues and characterized with respect to their amino acid sequences. So far, these studies have indicated that the gut-brain peptides from the two tissues have identical amino acid sequences. That of NPY, however, is clearly different from its intestinal counterpart, PYY, although both peptides possess identical N- and Cterminal amino acids and have the same number of amino acid residues. An immunohistochemical study has indicated that PYY may be absent from the brain 16. We have also been unable to detect PYY in the brain, nor has the brain peptide NPY been detected in the intestine by chemical assay and HPLC techniques. It is therefore possible that NPY is found only in nerve cells and PYY only in endocrine cells.

NPY seems to be present in large quantities in the brain, at concentrations even higher than those of VIP17. Clearly, it will be of interest to determine whether NPY is a neurotransmitter or neurohormone and to discover its physiological role(s) in the central nervous system.

This work was supported by grants from the Swedish Medical Council (project 13X-1010) and the Nordisk Insulinfond.

Received 7 December 1981, accepted 24 February 1982

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Acetylcholinesterase forms in fast and slow rabbit muscle

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In the skeletal muscles of vertebrates, the level of acetylcholinesterase (AChE, EC 3.1.1.7.) may be either decreased (in rat) or increased (in chicken1 and rabbit2.3) after denervation. This enzyme is very polymorphic, however, presenting molecular forms which differ in their cellular localization and physiological regulation. The different AChE molecules may be classified as globular forms (monomers G1, dimers G2 and tetramers G₄) and asymmetric or collagen-tailed forms (containing one, two or three tetramers: A4, A8 and A12)4. The distribution of the collagen-tailed and globular forms along the muscle fibres varies according to the animal species and physiological state: in normal adult rat muscle, the A12 form, which represents the major collagen-tailed form, is localized exclusively in the endplate region⁴⁻⁶, whereas in rat embryo⁷ and human muscle8 this form is distributed over the entire muscle fibre. The presence of collagen-tailed AChE has, however, been considered as an indicator of neuromuscular interactions because its appearance during embryogenesis coincides with the establishment of neuromuscular contacts^{6,9} and because, after denervation, the A12 form disappears from rat5,6 chicken10,11 muscles. Here we report that this form in fact increases markedly in a slow twitch oxidative muscle of the rabbit after denervation. The regulation of asymmetric-and particularly A12-forms of AChE thus depends dramatically on the species and on the nature of skeletal muscle.

One of the large muscles of the rabbit leg, the semimembranosus, consists of two very distinct and remarkably homogeneous parts12,13: the external region (region I) contains fast twitch type II glycolytic fibres and the internal conoidal bundle (region II) contains only slow twitch type I oxidative fibres (Fig. 1a) (P.V. and F.B., in preparation). Figure 2 illustrates an analysis of the AChE content of endplate-containing and endplate-free segments of these two muscle regions. In region I, the aneural sections contain very little AChE activity, while in region II, the specific activity is about half that of the endplatecontaining segments. In both fast and slow muscle regions, however, the collagen-tailed forms are preferentially, or exclusively, localized in the endplate-containing segments (the small proportion which is observed in the aneural samples may be due to the presence of a few endplates which have not been detected). In addition, the globular forms are also concentrated in the neural segments.

The semimembranosus muscle was usually denervated by removing a segment of the corresponding sciatic branch (the rest of the leg remaining mobile). After denervation, we noted a marked atrophy particularly affecting region I of the muscle (Table 1) and, by 2 months, an extensive infiltration of fatty inclusions. Although the red colour of the conoidal region II became paler, the two parts of the muscle remained very distinct. Figure 1b shows the histochemical appearance of the muscle after prolonged denervation. The slow type I fibres of region II are markedly atrophied and keep their acid-stable ATPase properties, whereas the fast type II fibres of region I are more heterogeneous, both in size and ATPase activities.

Table 1 shows that after denervation, AChE activity and acetylcholine receptors (AChR) rose to very high levels, reaching similar values in the two muscle regions at 2 weeks. The receptor level then continued to increase in region I, which became

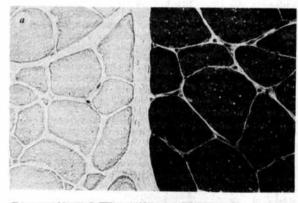
extensively atrophied. Although the receptor level appeared to decrease in region II, it remained at the high level characteristic of denervated muscle, and it seems unlikely that this interior muscle bundle could be re-innervated while the external mass of the muscle was not. Indeed, we never observed any sign of re-innervation in our experiments.

In denervated muscles, the AChE activity increased markedly, reaching about 10 times the original level in both muscle regions 1 month after the operation. It then continued to increase in region I, but decreased slightly in region II. After very long denervation periods (6 months), the slow conoidal bundle (region II) retained its characteristics, but the fast external part (region I) presented a very profound fatty degeneration, and no longer contained significant AChE activity.

Figure 3 illustrates the changes occurring in the composition of AChE molecular forms in regions I and II after denervation. The proportions of the various molecular forms were estimated from the sedimentation patterns, and used to determine their specific activities (see Table 2). In region I, the smaller globular forms increase after denervation, while the collagen-tailed forms decrease gradually, essentially disappearing 1 month after the operation. They reappear later in significant proportion (Fig. 3).

Region II follows a very different evolution: there is a marked increase of the collagen-tailed forms, both in proportion and in absolute level, which is maintained for at least 2 months.

We obtained very similar results when the sciatic nerve was transected near its root, paralysing the whole leg. Thus, a passive mechanical activity of the denervated muscles does not modify their evolution markedly.



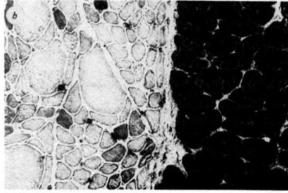


Fig. 1 Histochemical characterization of normal and denervated fast and slow regions of the rabbit semimembranosus muscle. Transverse sections of normal (a) or denervated (3 months) (b) semimembranosus muscle showing the limit between the external rapid part (region I, left) and the internal slow conoidal bundle (region II, right) stained for myofibrillar ATPase at pH 4.15 (×160). The light-coloured fibres of region I are fast-twitch type II, and the darkly stained fibres of the conoidal region II are slow-twitch type I. Cross-sections 14 μm thick were cut at $-15\,^{\circ}\mathrm{C}$ and stained for myofibrillar ATPase according to the method of Padykula and Herman 26 , modified by Guth and Samaha 27 . The staining procedure involved 12 min preincubation at 4 $^{\circ}\mathrm{C}$ in 1 M formic acid–NaOH buffer (pH 4.15), followed by 60 min incubation (37 $^{\circ}\mathrm{C}$) in 0.1 M barbital buffer, containing 18 mM CaCl $_2$ and 5 mM ATP (pH 9.4).

The present experiments show that the fast and slow parts of the rabbit semimembranosus muscle maintain their individuality for a very long time after denervation. Muscle types are thus very stable in the absence of innervation. We have in fact shown previously that distinctive characteristics may develop in nerve-free cultures of chick myoblasts obtained from muscles which in the adult become slow or fast¹⁴.

The difference in the evolution of the fast (region I) and slow (region II) parts of the denervated semimembranosus muscle is

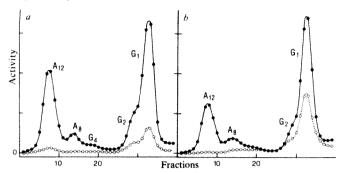


Fig. 2 Sedimentation profiles of AChE in endplate-containing and endplate-free sections of the fast (region I) and slow (region II) parts of the rabbit semimembranosus muscle, a. Region I; b. region II. . Endplate-containing segments; O, aneural segments. Small groups of fibres were teased from the two regions of the muscle, and endplates were visualized by the method of Koelle and Friedenwald, modified by Koenig and Rieger²⁸, which does not inactivate AChE. Neural and aneural segments could thus be dissected. They were homogenized in a glass-Teflon Potter homogenizer, with 10 vols of extraction medium containing antiproteolytic agents (NaCl 1 M, Triton X-100 1%, Tris-HCl pH 7, 10 mM, benzamidine 1 mM, bacitracin 1 mg ml⁻¹, Zymofren (aprotinin) 25 U ml⁻¹). Aliquots of the extracts were layered on 5-20% w/v sucrose gradients in saline detergent buffer (NaCl 1 M, MgCl₂ 50 mM, Triton X-100 1%, Tris-HCl pH 7 10 mM, bacitracin 0.1 mg ml⁻¹), and centrifuged in a Beckman SW 41 rotor, at 40,000 r.p.m. and 4 °C, for 20 h. *Escherichia coli* β -galactosidase (16S), beef liver catalase (11.3S) and calf intestine alkaline phosphatase (6.1S) were included as internal sedimentation standards, as described previously4. The apparent sedimentation coefficients of the AChE forms were approximately 16.2S (A_{12}) , 13S (A_8) and 8.5S (A_4) for the asymmetric forms, and 10.5S (G_4) , 5S (G_2) and 3S (G_1) for the globular forms. The asymmetric, collagentailed forms were characterized by their aggregation properties; they were no longer visible if the gradient was prepared without NaCl. The specific activity of AChE in aneural segments of region I was very low (0.8 nmol per min per mg protein), compared with that of endplate-containing segment (5.7 nmol per min per mg protein). In the case of region II, the specific activities of aneural and neural segments were, respectively, 3 and 7.6 nmol per min per mg protein. AChE activity is plotted on an arbitrary scale but the profiles corresponding to the neural and aneural samples of each region have been normalized so that the activities of each form in the two segments are directly comparable.

particularly remarkable with respect to the collagen-tailed forms of AChE. In region I, the localization and evolution of AChE appear somewhat similar to those previously observed in chicken fast-twitch muscle: the activity is highly concentrated at the endplates in the normal muscle¹⁵, and after denervation there is a concomitant decrease of the A₁₂ form and increase of the small globular forms. In contrast, the dramatic increase in the A₁₂ collagen-tailed form which occurs in the denervated region II of semimembranosus disagrees with previous observations in other species, which suggested that this form disappeared from all skeletal muscles after denervation, even when the overall level of AChE was elevated. This increase shows that the continued presence of the nerve is not necessary for the biosynthesis of collagen-tailed AChE forms, a conclusion reinforced by the reappearance of the A₁₂ form in the fast region (I) of the muscle after prolonged denervation.

In addition, the very high level of A_{12} , and to a lesser extent A_8 , in denervated region II indicates that these forms are probably not limited to the original sites of neuromuscular contacts. Indeed, we found that segments from 1-month denervated region II, corresponding to originally endplate-containing and endplate-free regions, contained exactly the same AChE activity with the same pattern of molecular forms, in particular a high proportion of A_{12} form.

The histochemical analysis of Tennyson et al.³ showed that after denervation, the AChE activity increases mostly in the extrajunctional part of the muscle fibres, notably at intracellular sites, and is largely associated with the sarcoplasmic reticulum. It is thus very likely that a fraction of the molecular forms synthesized in such conditions, including the collagen-tailed forms, remain intracellular, associated with the reticulum. One example of such an intracellular localization of collagen-tailed forms has been reported in the case of motor nerves, where these molecules are transported by fast axonal flow¹⁶.

The present observations do not contradict the hypothesis that neuromuscular interactions are required to trigger the biosynthesis of the collagen-tailed forms: Koenig and Vigny¹⁷ found that muscle cells from 13-day-old rat embryos only synthesize the A₁₂ form when cultured in the presence of neurones, but that myoblasts obtained from 18-day-old embryos possess the capacity to synthesize this form by themselves. Rubin et al. ¹⁸ have also demonstrated an induction of the A₁₂ form in cultures of chick myotubes, by synaptic contacts with cholinergic neurones, while Kato et al. ¹⁹ found that this enzyme is synthesized in cultures of muscle cells isolated from innervated muscles of 11-day-old chick embryos. These findings

Table 1 Evolution of AChE and acetylcholine receptors in fast (I) and slow (II) regions of rabbit semimembranosus after denervation

		Control muscle	2 Weeks denervated muscle	1 Month denervated muscle	2 Months denervated muscle
Fast twitch	Total weight of muscle (g) AChE (nmol per min per mg protein) AChR (pmol per mg protein)	20.8	11.1	6.0	5.8
semimembranosus		2.2	13	29.3	62.1
region (I)		0.01-0.02	0.34	0.47	0.76
Slow twitch	Total weight of muscle (g) AChE (nmol per min per mg protein) AChR (pmol per mg protein)	2.23	1.76	1.10	1.42
semimembranosus		4.3	15.7	41.6	30
region (II)		0.01–0.02	0.34	0.19	0.15

The rabbits used were New Zealand white females weighing 2.800-3.100 kg. The weight of the muscle has been normalized for 3 kg body weight. The specific activity of AChE was determined in muscle extracts obtained in a detergent saline buffer containing antiproteolytic agents (Tris-HCl, pH 7 10 mM, EDTA 10 mM, NaCl 1 M, Triton X-100 1%, benzamidine 1 mM, Zymofren (aprotinin) 25 U ml⁻¹, bacitracin 1 mg ml⁻¹. AChE activity was determined by the spectrophotometric method of Ellman and colleagues²⁴ as described previously⁴ in the presence of 10⁻⁵ M ethopropazine, an inhibitor of nonspecific cholinesterase. The extract was preincubated for at least 1 h in the reaction mixture in the absence of the substrate acetylthiocholine, in order to avoid interference with the reaction of sulphydryl groups in the extract with 5, 5'-dithio-bis(2-nitrobenzoic)acid (DNTB). We verified that the preincubation of AChE with DNTB did not modify its activity. The activity of the 'nonspecific' cholinesterase (EC 3.1.1.8.) was negligible in the muscle extracts, even after denervation. AChR were assayed by measuring the binding of ¹²⁵I-labelled bungarotoxin (NEN) (10-20 µCi per µg) to a membrane preparation obtained in the following manner. A sample of finely minced muscle was homogenized with a Polytron homogenizer in 10 vol of buffer (Tris-HCl, pH 7.5, 50 mM, EDTA 3 mM, EGTA 1 mM, phenylmethylsulphonylfluoride 0.1 mM, aprotinin (Sigma) 5 trypsin inhibitor units per 1). The homogenate was filtered through a nylon sieve to eliminate the fibrous material. After centrifugation at 20,000g for 30 min, the pellet was resuspended in 5 vol of buffer and aliquots of this particulate suspension were incubated for 2 h at room temperature with a saturating concentration of labelled bungarotoxin (8 nM). In control samples a 100-fold excess of cold erabutoxin was added 1 h before the labelled toxin. After incubation, the suspension was diluted with 2 ml cold buffer, centrifuged and the pellet was resuspension was collined buffer, before coun

Table 2 Evolution of AChE molecular forms in denervated rabbit semimembranosus muscle

		Control muscle	2 Weeks denervated muscle	1 Month denervated muscle	2 Months denervated muscle
Fast twitch semimembranesus region Specific activity (nmol per min per mg total protein) and proportion of total activity	A ₁₂ (16.2S)	0.8 (36%)	0.6 (4.5%)	(<0.5%)	1.2 (2%)
	A ₈ (13S)	0.2 (10%)	0.4 (2.5%)	(<0.5%)	07 (1%)
	G ₄ (10.5S)	0.1 (4%)	0.5 (5%)	3 2 (11%)	76 (12%)
	G ₂ (5S)	0.1 (4%)	0.3 (3%)	1.8 (6%)	3.6 (6%)
	G ₁ (3S)	1.0 (47%)	11.0 (84.5%)	24.2 (83%)	48.4 (78%)
Slow twitch semimembranosus region Specific activity (nmol per min per mg total protein) and proportion of total activity	A ₁₂ (16.2S)	0 4 (9.5%)	1.9 (12%)	10.8 (26%)	7.2 (24%)
	A ₈ (13S)	0.4 (10%)	0.9 (6%)	3.1 (7.5%)	3.8 (13%)
	G ₄ (10.5S)	0.2 (5%)	1.9 (12%)	7.5 (18%)	3.8 (13%)
	G ₂ (5S)	0.1 (2%)	0.3 (2%)	0.7 (2%)	0.8 (25%)
	G ₁ (3S)	3.0 (69%)	10.7 (68%)	19 6 (47%)	14.1 (47%)

The proportions of the various molecular forms were estimated from the sedimentation profiles (Fig. 3), and the specific activity of each form was then obtained from the total AChE activity, given in Table 1. Although the minor asymmetric A4 form represents a significant component in some cases (for example, ~5% in the central slow region), its contribution is generally negligible and is not listed.

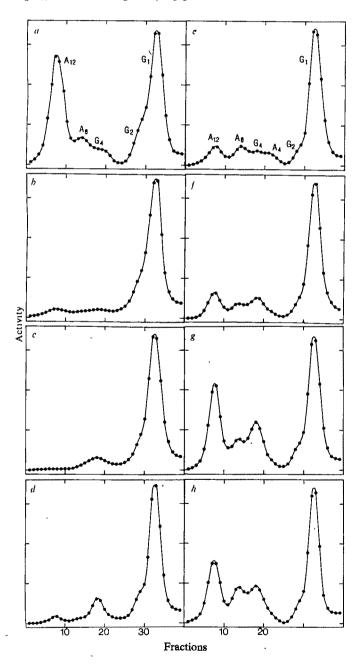


Fig. 3 Sedimentation profiles of AChE in the fast (region I) and slow (region II) parts of the normal and denervated rabbit semimembranesus muscle. a-d, region I; e-h, region II The muscle was finely minced with a scalpel; aliquots of the mince were crushed into a powder in liquid nitrogen, homogenized in a Teflon Potter homogenizer and the extracts were analysed as indicated in Fig. 2. The proportions and specific activities of the different forms of AChE are reported in Table 2

suggest that nerve-muscle interactions occurring in vivo may leave permanent instructions allowing the assembly of collagentailed AChE in culture. The presence of the A₁₂ form in cultures of a transformed murine muscle line19 indeed shows that this capacity can be transmitted indefinitely.

It seems likely that the proportion of the different molecular forms of AChE observed in normally innervated muscles in vivo corresponds to specific physiological requirements; the different forms possess distinct interaction properties4 and their proportions therefore control the spatial distribution of the enzyme within synaptic structures. For example, the ionic interactions of the collagen-tailed forms²⁰ may anchor them in the intersynaptic basal lamina²¹. These forms might be specifically required in neuromuscular junctions at which transmission is effected by impulses of very short duration: in normal adult chickens, the A₁₂ form becomes largely predominant in the phasic, focally innervated posterior latissimus dorsi muscle but disappears in the tonic anterior latissimus dorsi muscle²².

In conclusion, the nerve seems to exert a twofold influence on the muscle: an initial induction effect which allows the synthesis of collagen-tailed AChE and possibly determines the type of fibre—although this can be modified, for example, by reinnervation with a different neurone—and a functional regulation which requires its continued presence. This second effect is probably mediated in part through the muscle's activity23, perhaps by the intracellular level of cyclic GMP¹⁸. Denervated muscles apparently escape this physiological regulation, producing either increased or decreased levels of AChE, with highly variable patterns of molecular forms, depending on the species, and on the type of muscle.

We thank Marylène Rostagno and Pierre Allemand for technical assistance, and Dr. Robert Oswald for help with the acetylcholine receptor assays. This work was supported by the Institut National de la Recherche Agronomique, the Centre National de la Recherche Scientifique and by grants 80.7.0353 and 79.7.1059 of the Délégation Générale à la Recherche Scientifique et Technique.

Received 23 November 1981, accepted 2 March 1982

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A rat monoclonal antibody against mouse α and β interferon

of all molecular weight species

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Monoclonal antibodies have proven to be invaluable reagents for the characterization and purification of human interferons (IFN). Hybridoma lines secreting monospecific antibodies against human IFN- α and IFN- β have been isolated, and these antibodies have shown no cross-reactivity between the two interferons¹⁻⁴. Murine interferons, when analysed by polyacrylamide gel electrophoresis, have two major migration patterns, one in the 28,000-35,000 (28-35 K) molecular weight (M_r) region and the second in the 22 K region⁵⁻⁸. Based on some homology of the N-terminal amino acid sequence with human interferons, the 28 K and 35 K species are classified as mouse IFN-B and the 22 K species as mouse IFN- α^9 . Minor species have also been described but their relationship to IFN- α and IFN- β is unknown^{10,11}. We have now established a stable hybridoma line secreting rat antibodies to mouse virus-induced interferon. The monoclonal antibodies are of the IgG class, with binding activity to all the different M, specied, as demonstrated in an enzyme-linked immunosorbent assay (ELISA) and by affinity chromatography. In addition, the antiviral activity of all the M_r species was neutralized by the antibody but the neutralizing activity was much weaker than the binding activity. This indicates the presence of a common antigenic site for all mouse α and B interferon species.

The interferon used for immunization was elicited with Newcastle disease virus (NDV) in monolayer cultures of Swiss mouse C-243 cells as described elsewhere 10. Purification was performed by two-step affinity chromatography on poly(U)-Sepharose and anti-interferon antibody-coupled Affigel-10 columns. The antibody used for this purification consisted of the globulin fraction of serum from a goat immunized with NDVinduced C-243 cell mouse interferon. The specific activity of the purified interferon was 2.4 × 10° U per mg protein (all interferon units are expressed as international reference units) and its electrophoretic purity was assessed on 15% polyacrylamide slab gels in the presence of SDS in a Tris-glycine buffer system⁸. The biological activity of interferon preparations was determined by measuring the protection from the cytopathic effect of vesicular stomatitis virus on L929 cell monolayers.

An 8-week-old female rat of the LOU strain received $5 \times$ 10⁷ U of purified interferon in complete Freund's adjuvant subcutaneously (s.c.) and intraperitoneally (i.p.). This corresponds to 50 μ g of interferon if calculated with a specific activity of 2.4×10^9 U (ref. 8). A second set of injections (s.c. and i.p.) with 2.5×10^7 U (25 μ g) of purified interferon was given 3 weeks later in incomplete Freund's adjuvant. After 3 weeks the animal received 0.5×10^7 U (5 µg) of purified interferon i.p. on 3 consecutive days. The rat produced serum antibodies against interferon, with a neutralizing titre of 1/8,000 as measured against 4 interferon units. It was killed 2 days after the last injection and spleen and mesenteric lymph nodes were aseptically removed. The cells were fused with LOU-derived 210RCY3-Agl.2.3 rat myeloma cells¹² according to the protocol of Köhler and Milstein¹³ modified by Fazekas et al.¹⁴. After fusion, the cells were cultured in Falcon Microtest plates at a density of 8×10^5 spleen cells per well on mouse peritoneal macrophage feeder layers in hypoxanthine-aminopterin-thymidine medium; 960 wells were seeded. Supernatants of growing hybridoma colonies were tested for binding activity using an ELISA. Microelisa plates (Dynatech, M 129B) coated with purified interferon were filled with 50 µl of hybridoma supernatant and kept at 37 °C for 30 min. After washing, horseradish peroxidase-conjugated rabbit anti-rat immunoglobulin preparations (Cappel Laboratories) were used as indicator antibody in 1:200 dilution in phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA) and 1% Tween 20. After 30 min incubation at 37 °C the wells were washed and for the colour reaction, 100 µl of 25 µg o-phenylenediamine per ml of 10 mM phosphate buffer, pH containing 0.02% H₂O₂ (30%) were added. The reaction was terminated after 20-30 min with 50 µl of 3M HCl and absorption was read at 492 nm. Of the 960 wells seeded, 250 were positive for hybridoma growth, and the supernatants of 17 of these gave a positive ELISA reaction (twice the background reading), but only one (clone C5A) was found to be stable after two subclonings. Cells from wells containing supernatant active in ELISA were immediately subcloned by limiting dilution and supernatants from subclones tested. In this way a stable hybridoma line secreting monoclonal antibodies binding to electrophoretically pure interferon was established. The antibody belongs to the rat IgG class, as determined by immunoprecipitation, gel filtration and electrophoresis on 15% polyacrylamide gel in the presence of 0.1% SDS and 1% \(\beta\)-mercaptoethanol. The secretion of antibodies was checked regularly and the cell line found to be stable for

For mass production of antibodies, adult male rats (LOU) were injected i.p. with 2 ml of Pristan mineral oil and 1 week

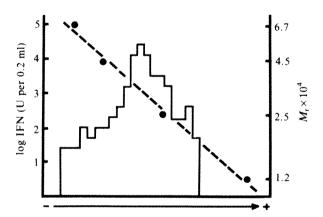


Fig. 1 Electrophoretic profile of interferon activity eluted from polyacrylamide gels after affinity chromatography on monoclonal antibodies fixed to Affigel-10 (Biorad). To prepare the column, ammonium sulphate-precipitated antibodies derived from 50 ml ascites fluid was coupled to the Affigel in 100 mM phosphate buffer, pH 7, at 4 °C. Free binding sites were saturated with ethanolamine and the column washed with a cycle of 100 mM phosphate buffer pH 7 and then with a cycle of 100 mM citrate buffer, pH 2.2. Interferon purified on poly(U)-Sepharose $(7.7 \times$ 10⁵U) was applied to the column. No interferon activity was recovered in the flowthrough. After a washing step, the bound interferon was desorbed with citrate buffer, pH 2.2. The peak fractions were pooled, dialysed against 125 mM Tris buffer, pH 6.8, containing 1% SDS then concentrated on an Amicon PM10 membrane and boiled with 1% β-mercaptoethanol for analysis on a 15% polyacrylamide gel in the presence of 0.1% SDS. Molecular weight markers consisted of BSA (67,000), ovalbumin (45,000), chymotrypsinogen (25,000) and cytochrome c (12,500). For the determination of interferon activity, 2-mm slices were cut and eluted overnight at 4 °C.

Table 1 Neutralization of the antiviral activity of the different M_r species of murine interferon

_	log :	IFN titre
$M_r(\times 10^3)$	Control	+ Antibody
65	3.28	2.68
35	5.38	4.78
28	4.48	3.88
22	3.28	2.98
15	2.38	2.07
Unfractionated IFN	5.88	5.28

Interferon purified on poly(U)-Sepharose⁸ was electrophoretically separated on 15% polyacrylamide gels in the presence of 0.1% SDS and 1% β -mercaptoethanol. 1-mm slices of the gel were eluted overnight in 0.2 ml of Tris-SDS 0.1%. The antiviral activity of the eluates was determined in the presence or absence of 2.5 µl of antibody-containing ascites fluid at each dilution of the samples and expressed as \log_{10} of the interferon titre. Comparable results were obtained when the antibody was prepared from supernatants of hybridoma cultures. The results of four different neutralization experiments indicated no significant difference in the degree of neutralization of the various M_r species. Supernatants of myeloma cultures, concentrated and treated like the hybridoma supernatants, were without effect.

later with $5-10\times10^6$ hybridoma cells, also i.p. One to two weeks later ascites fluid was recovered from the peritoneal cavity and IgG purified by ammonium sulphate precipitation, sometimes followed by gel filtration on Sephacryl-S300. The interferonbinding activity in ELISA from the ascites fluid was 1,000-fold higher than that from culture supernatants.

When crude mouse interferon, consisting of a mixture of all $M_{\rm r}$ forms, was tested in the presence of purified monoclonal antibody, the antiviral activity was significantly reduced. However, the neutralizing titre was considerably lower than the binding titre, since an antibody preparation giving a positive ELISA reaction at a dilution of 1/12,800 completely neutralized 4 U of mouse interferon only at a dilution of 1/32, or lower.

Anti-interferon antibodies from culture supernatant were retained by protein A-Sepharose in binding conditions via the Fc part of the IgG molecule. Crude interferon was applied to the column after extensive washing, antigen-antibody complexes were desorbed. The elution pattern of interferon activity desorbed at pH 3, after electrophoresis on 15% polyacrylamide gel in the presence of SDS, shows that the protein A-Sepharose coated with the monoclonal antibodies retained both IFN-α (22 K) and IFN- β (28-35 K). Insufficient interferon was used in this experiment to allow detection of the 65 K and 15 K species. Additional evidence for binding to all interferon species was then obtained by affinity chromatography on monoclonal antibodies bound to Affigel-10. In these conditions, there was complete retention of NDV-induced C-243 interferon and the bound interferon could be desorbed by lowering the pH with 0.1 M Na-citrate buffer. Because the major α and β interferon species are present in about equivalent amounts in our crude C-243 cell interferon preparation, as shown by neutralization with anti-mouse IFN- β serum, total retention by the column is indicative of affinity for all M_r forms. This was confirmed by an electrophoretic analysis of the interferon desorbed from the column which showed that the major M_r forms were present in the desorbed material (Fig. 1). The minor 15 K species was not recovered from the gel, but affinity of the antibody for this species was demonstrated in the following experiments.

To determine the capacity of the antibody to neutralize all the different mouse interferon species, a preparation of NDVinduced C-243 interferon, prepared as described previously 10, was purified and concentrated on poly(U)-Sepharose. An aliquot of the peak fraction, with a titre of 2.4×10^7 U, was dialysed overnight against sample buffer (0.125 mM Tris pH 6.8, β -mercaptoethanol 1%, SDS 0.1%) and after boiling for 2 min, electrophoresed on a 15% polyacrylamide slab gel as described previously⁸. The gel was cut into 1-mm slices which

were eluted overnight in 0.2 ml of Tris-SDS 0.1% per slice. Titration of the interferon activity of all fractions in the presence or absence of themonoclonal antibody revealed the presence of the two major peaks, one at 28-35 K (murine IFN- β) and one at 22 K (murine IFN- α) and the two minor peaks of 65 K and 15 K. The antiviral activity of all the fractions was reduced by a factor of two to four in the presence of the antiserum: the results for the peak fractions are summarized in Table 1. Affinity of the antibody for all the M_r forms was then confirmed in an ELISA assay, in which a 50-µl aliquot of each fraction was left to adsorb to Microelisa plates (Dynatech M129) overnight at room temperature. The reaction was then carried out as described above, using antibody obtained from ascites fluid. A positive reaction was scored in all the wells that had received the fractionated interferon, from 65 K to 15 K down.

All previously described monoclonal antibodies against human interferon show specificity for either IFN- α or IFN- β . Recently, a monoclonal rat antibody against mouse IFN-B has been obtained that did not bind mouse IFN- α^{15} . Therefore, it is rather surprising that the first monoclonal antibody we obtained after immunizing a rat showed no specificity in binding to a particular mouse interferon species but instead binds to all of them. Antigenic differences between mouse IFN- α and IFN- β have been described by Yamamoto and Kawade¹⁶, who immunized rabbits against the fast- and slow-moving components of NDV-induced mouse L-cell interferon. The antisera they obtained showed some cross-reactivity as measured by binding of interferon to immobilized antibody columns and the authors attributed this to possible crosscontamination of the interferons used for immunization.

The results with the monoclonal antibody presented here suggest that the antisera of Yamamoto and Kawade may well have contained cross-reacting antibodies. Tryptic digests of the high M_r species (40 K, corresponding to mouse IFN- β) and the low M_r species (24 K, corresponding to mouse IFN- α) of mouse L-cell interferon have revealed the presence of a common polypeptide structure¹⁷ and regions of homology have also been found in the amino acid sequence of human α and β interferons 18. As indicated by the results of the binding and neutralization experiments with the rat monoclonal antibody that we have obtained, at least one antigen site is shared by all M_r forms of mouse interferon. This may be a highly conserved region of some importance for the activity of the different interferon species.

We thank J. Hurst and L. Eusèbe for technical assistance, T. Ternynck for introducing us to the ELISA technique, Dr E. Günther for the gift of LOU rats, and Dr L. Kronenberg for providing us with anti-mouse IFN- β rabbit serum. D.M. was supported by a fellowship of the Deutsche Forschungsgemeinschaft and is at the Deutsches Krebsforschungszentrum, Heidelberg. R.K. is at the Friedrich Miescher Institut, Tübingen. This work was supported by the Foundation pour la Recherche Médicale Française and the Ligue National Française contre le Cancer.

Received 14 December 1981; accepted 5 March 1982.

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Monoclonal antibodies against unique *I*-region gene products expressed only on mature functional T cells

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The immune response (I) region of the murine major histocompatibility complex (MHC) regulates specific immune responses against antigens1 and encodes cell-surface molecules designated as Ia antigens². Ia antigens are generally detected on B cells and macrophages, and have important roles in immunocyte interactions. On the other hand, it has been reported that some T cells and their products (antigen-specific T-cell factors) carry unique I region-encoded determinants which are different from B-cell Ia antigens³⁻¹⁰. As T-cell factors are known to mediate T-T and T-B cell interactions11, elucidation of such unique I-region determinants on T cells is obviously important for understanding the regulatory cell interactions of the immune response. However, this has long been hampered by the lack of reagents specific for the determinants and due to the small number of T cells expressing them. To overcome this difficulty, cell hybridization techniques have been used to develop T-cell hybrids which continuously express I-region products, and monoclonal antibodies specific for these determinants have been produced. Here we report the production of monoclonal antibodies which detect a unique set of I-region determinants expressed only on functional subsets of T cells and T-cell hybridomas.

The rationale used in this study was based on our previous findings that antigen-specific augmenting (T_*F) and suppressor (T_*F) factors produced by immune splenic T cells of mice carry *I*-region determinants encoded in the *I-A* and *I-J* subregions of the MHC^{6,9}. Both these determinants are distinct from B-cell Ia antigens with respect to molecular size, composition, antigenspecific regulatory function and serological cross-reactive pattern. In addition, they possess antigen-binding sites with idiotypes and immunoglobulin variable region determinants ¹². These properties suggest that the Ia antigens associated with

T-cell factors may have prototypic structures entirely different from those of conventional B-cell Ia antigens, which consist of α and β polypeptides¹³.

We have obtained T-cell hybrids which produce antigen-specific T_*F and T_*F with I region-encoded determinants^{14,15}. Such hybrids are apparently ideal tools for screening monoclonal antibodies reactive to putatively unique T-cell Ia antigens. We therefore produced monoclonal antibodies which react specifically with these determinants expressed on T-cell hybrids. Spleen cells of A.TH (H-2¹²) mice immunized with A.TL (H-2^{t1}) lymphoid cells were fused with P3-X63-Ag-8-653, a 8-azaguanine-resistant plasmacytoma of BALB/c mouse origin. Strains A.TH and A.TL differ only in the I and S regions of the MHC, thus spleen cells of immune A.TH mice should contain B cells specific for I-region products of $H-2^k$ haplotype. The fused cells were selected in hypoxanthine-aminopterinthymidine medium, and were cloned by limiting dilution. The culture supernatants of cell lines were screened for antibody activity against a T-cell hybrid (FL10) of A/J mouse origin, which had been shown to produce T_aF specific for keyhole limpet haemocyanin (KLH) associated with a unique product of the I-A subregion of $H-2^a$ (ref. 14).

The supernatants of 31 out of 201 clones were reactive with FL10 in a microcytotoxicity assay and in fluorescence-activated cell sorting (FACS) analysis of two fusion experiments. Those clones which stained parental thymoma, BW5147, and Ianegative T-cell hybrids were excluded. They were further screened with spleen cells of nude C3H mice $(H-2^k)$, and only those which did not react with B cells and macrophages were selected. Eleven clones were finally established using the criteria that their antibodies reacted with FL10 but not with BW5147 and $H-2^k$ B cells (Table 1).

The monoclonal antibodies were tested further with enriched antigen-binding T cells of A/J $(H-2^a)$ mice prepared by adsorption of KLH-primed splenic T cells to antigen-coated plastic dishes¹⁶; A/J mice produce augmenting T cells expressing $I-A^k$ but are unable to make $I-J^+$ suppressor T cells on immunization with KLH⁹. All the antibodies reactive to FL10 were, in fact, strongly cytotoxic for the enriched KLH-binding T cells of A/J but were unable to kill non-antigen binding T cells (Table 1). They were also unable to kill normal splenic and thymic T cells of both A/J and C3H, suggesting that the proportion of T cells expressing these I-region determinants is too small to be estimated by the usual cytotoxicity test. The

Table 1 Cytotoxicity of monoclonal antibodies for $I-A^+$ T-cell hybridoma FL10 and lymphocyte subsets

			% Lysis of tare	ets by complement-	dependent cytotoxic	itv
Monoclonal antibody	Isotype	FL10	Thymocytes	C3H nu/nu spleen cells	KLH-binding splenic T cells of A/J	KLH-non-binding splenic T cells of A/J
1L9	IgM	52	0	1	21	0
2L2	IgM	65	0	1	38	0
3L1	IgM	44	0	0	21	0
7L114	IgM	19	·· 0	0	36	0
14P	IgM	33	0	0	15	0
21L27	IgM	40	0	0	16	0
34L5	IgM	25	0	0	20	0
88L7	ND	31	0	0	21	0
119L2	IgG	27	0	0	12	0
148L4	IgG	32	0	0	22	0
164L1	ND	14	0	0	25	0
A.TH anti-A.TL		60	0	80	30	0

Screening of monoclonal antibodies using the T-cell hybrid, FL10, and KLH-binding T cells. Culture supernatants of hybrid clones were tested for reactivity to various preparations of normal cells, as well as to the Ia-positive T-cell hybrid, FL10, by the dye exclusion cytotoxicity test. 20 μ l of culture supernatant were mixed with the same volume of cell suspension $(2 \times 10^6 \text{ ml}^{-1})$ and incubated at room temperature for 15 min. After washing, 20 μ l of selected rabbit complement were added, and the mixture was incubated at 37 °C for 30 min. Per cent lysis was calculated using the formula $(a/b)/(100-b)\times 100$ where a= percentage of cells stained by Trypan blue after incubation with the culture supernatant and complement, and b= percentage of cells stained after incubation with complement alone. KLH-binding T cells of A/J mice were prepared from spleen cells of KLH-hyperimmunized A/J mice. Spleen cells were first layered onto plastic dishes coated with purified anti-mouse immunoglobulin to remove B rigorous washing with chilled medium, and were used as KLH-binding T cells. Nonadherent T cells were used as control. Isotypes of the monoclonal antibodies were determined by double immunodiffusion with purified anti-isotype antibodies. ND, not determined.

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T-cell specificity of the monoclonal antibodies was confirmed by absorption of culture supernatants with A/J splenic B cells obtained by treatment with a rabbit anti-brain antiserum and complement (C): after absorption, the cytotoxic activity for FL10 remained intact, whereas absorption with unseparated spleen cells removed the antibody activity (Table 2). This result indicates that some T but not B cells express the *I*-region determinants detected by our monoclonal antibodies.

Haplotype and *I*-subregion specificities of the monoclonal antibodies were determined by absorption with splenic T cells of different strains (Table 2). Absorption with spleen cells of A/J, A.TL, B10.BR $(H-2^k)$ and B10.A(4R) eliminated the ability to kill FL10, whereas absorption with A.TH and B10.S $(H-2^i)$ cells failed to do so. Cytotoxicity for FL10 was not removed by absorption with B10 $(H-2^b)$ spleen cells, except for one (21L27) which showed a definite cross-reactivity with the $H-2^b$ haplotype. These results indicate that the antibody specificity is directed primarily at the product of the I-A subregion of $H-2^k$. All the monoclonal antibodies were entirely refractory in their cytotoxicity for virus-producing T lymphomas such as E&G2 $(H-2^d)$, EL-4 $(H-2^b)$ and AKSL-2 $(H-2^k)$, which excludes the possibility that they are antiviral antibodies.

Having established that these monoclonal antibodies consistently react with *I-A*⁺ FL10, we performed experiments to determine whether they are actually detecting *I*-region determinants selectively expressed on certain functional T-cell

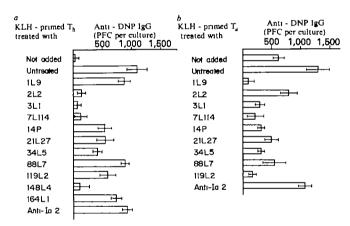


Fig. 1 a, Effect of monoclonal antibodies on the KLH-specific helper T cell (Th). DNP-primed B cells were obtained from spleen cells of DNP-KLH-primed mice after treatment with rabbit antibrain antibodies and complement. They were co-cultured with KLH-specific syngeneic helper T cells obtained by incubating KLH-primed spleen cells in plastic dishes coated with rabbit anti-mouse immunoglobulin (anti-MIg). The KLH-primed splenic T cells were treated with monoclonal antibodies (~0.1 μg antibody) and complement before the co-culture with B cells. Anti-DNP IgG-forming cells (plaque-forming cells, PFC) were counted 5 days after the start of cultivation. Note that treatment with some of the antibodies (2L2, 3L1, 7L114 and 148L4) resulted in complete loss of helper activity. Other monoclonal antibodies show variable degrees of effects on helper activity. b, Effect of monoclonal antibodies on the KLH-specific augmenting T cell (T₂). T cells of KLH-primed C3H mice were obtained by incubating the spleen cells in anti-mouse immunoglobulin-coated plastic dishes. They were then treated with monoclonal antibodies and complement, and the live cells were recovered by centrifugation on a layer of fetal calf serum. TaF was extracted from the cells by repeated freezing and thawing followed by ultracentrifugation at 40,000g for 1 h. The cell-free extracts corresponding to 3×10^6 original T cells were added to the test culture of DNP-KLH-primed spleen cells 2 days after the start of cultivation. This procedure was found to be suitable for detecting the TaF activity without much effect due to T₂F⁷. Anti-DNP IgG PFC were estimated 5 days after the onset of culture. Note that all monoclonal antibodies had an effect on the T_aF activity, except anti-Ia.2 which is specific for the B-cell Ia antigen. The suppression of the antibody response in some cases is probably due to the predominant suppressor effect after selective eliminat. on of augmenting T cells (see text).

subsets. We used two different methods: (1) the KLH-primed C3H ($H-2^k$) helper T cells were treated with monoclonal antibody and complement, and the cells were co-cultured with syngeneic B cells primed with a hapten, 2,4-dinitrophenyl (DNP), to examine the effects on carrier (KLH)-specific helper T cells; and (2) the KLH-primed splenic T cells were treated with monoclonal antibody and complement, and KLH-specific T_aF was extracted from the cells to assay residual T_aF activity by addition to the *in vitro* secondary anti-DNP antibody response.

Figure 1a illustrates the ability of some of the monoclonal antibodies to eliminate the helper activity of carrier-specific splenic T cells. Figure 1b demonstrates that many of these monoclonal antibodies are also effective in eliminating T_aFproducing cells. In some cases, the response was greatly suppressed, suggesting that the selective killing of T.F producer resulted in the predominance of remaining suppressor effects. A well-defined monoclonal antibody against a B-cell Ia antigen (anti-Ia.2) was found to be ineffective in eliminating both the helper and augmenting T cells. The ability of the monoclonal antibodies to remove helper and augmenting T cells did not always correlate; as some of the antibodies could kill only T₂F producer, there was an apparent reduction in the net helper activity as demonstrated in Fig. 1a. It was further confirmed that immunoadsorbents of at least three of the monoclonal antibodies (2L2, 3L1 and 14P) tested were able to absorb FL10derived T_aF (data not shown).

The results indicate that there exist I-region-encoded determinants expressed only on helper and augmenting T cells as detected by our monoclonal antibodies. These determinants seem entirely different from conventional Ia antigens of B cells and macrophages, as none of our monoclonal antibodies reacted with these cell types. It is of interest that the determinants are the products of genes mapped to the I-A subregion, the chromosomal segment which was defined originally by the presence of the Ir-1 gene¹ and later by the Ia-1 locus controlling the B-cell Ia antigen². As none of our antibodies react with B cells, we presume that the I-A subregion carries genes which are selectively expressed on augmenting and helper T cells. In addition, we have developed several hybridomas producing antibodies against at least three discrete molecules on suppressor and helper (T_{h2}) T cells controlled by genes in the adjacent I-J subregion (A. Kurata et al., unpublished results). Thus, we suggest that there exists a set of multiple loci tandemly arranged on the I-region chromosomal segment which is expressed only on mature functional T cells. As some of these I-region gene

Table 2 I-A subregion assignment of two monoclonal antibodies by absorption with strains of various haplotype

Spleen cells used for absorption	% Lysis of 14P	f FL10 by 1L9
A/J (H - 2^a) A/J (isolated B cells) A.TH (H - 2^{t2}) A.TL (H - 2^{t1}) B10.BR (H - 2^k) B10.S (H - 2^t) B10.S (H - 2^t)	28 <10 22 34 <10 <10 <10 <30 30 32	24 <10 ND 30 <10 <10 <10 31

Culture supernatants of two representative clones, 1L9 and 14P, were absorbed with various doses of spleen cells from strains of different haplotypes, and were tested for their residual cytotoxic activity against FL10. The results given here are those obtained by absorption with $1-5\times10^6$ cells for 100 μ l of culture supernatant. B cells were isolated from A/J spleen cells by treatment with anti-brain antiserum and complement. Note that absorption with $I-A^k$ spleen cells invariably eliminated the cytotoxicity of monoclonal antibodies for FL10, whereas spleen cells having a haplotype other than $I-A^k$ were unable to absorb the antibody activity. One other monoclonal antibody (21L27) cross-reacted with $H-2^b$. Some others showed cross-reactivity with $I-J^k$ and $I-E/C^k$ products; B10.A(3R) and B10.A(5R), as well as B10.A(4R), could absorb the anti-FL10 activity. These will be described elsewhere.

products on T cells were originally defined as a component of antigen-specific T-cell factors11, that is, putative antigen receptors of T cells, biochemical and genetic studies of the molecules reactive to our monoclonal antibodies are required to define the T-cell unit responsible for antigen recognition. Furthermore, our results cast new light on the organization and function of MHC in relation to the genetic regulation of various T-cell functions. The reagents described here should be useful for re-examining the antigen receptors of T cells and the molecular mechanism of MHC-restricted cell interactions.

We thank Drs K. Okumura and M. Taniguchi for criticisms, and Ms Yoko Yamaguchi and Taeko Fukuda for secretarial and technical assistance. This work was supported by a grant from the Ministry of Education, Culture and Science of Japan.

Received 22 September 1981; accepted 9 March 1982.

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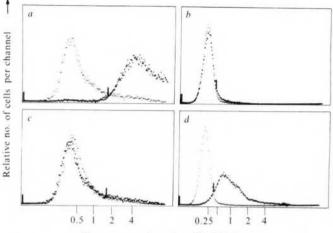
Structural homology of a macrophage differentiation antigen and an antigen involved in T-cell-mediated killing

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Two distinct murine cell-surface differentiation antigens, Mac-1 and LFA-1 (lymphocyte function-associated antigen 1), are compared here and shown to be related at the molecular level. Mac-1, defined by the M1/70 rat anti-mouse monoclonal antibody (MAb), is expressed on macrophages, natural killer cells and 50% of bone marrow cells, but not on B or T lymphocytes1-3. In contrast, the LFA-1 antigen, defined by the M7/14 rat anti-mouse MAb, is expressed on B and T lymphocytes and 75% of bone marrow cells, but not on thioglycollate-induced peritoneal exudate macrophages^{4,5}. MAb blocking studies suggest that LFA-1 participates in T-lymphocytemediated killing and T-lymphocyte antigen-specific responses^{4,5}. Mac-1 and LFA-1 have α-polypeptide chains of 170,000 and 180,000 molecular weight (M_r) , respectively, and both contain β polypeptides of 95,000 M_r . This similarity prompted us to investigate their relationship. Mac-1 and LFA-1 have distinct cellular distributions, MAb-defined antigenic determinants and α-subunits, but have highly homologous or identical β -subunits as shown by tryptic peptide mapping. Moreover, they share some common antigenic determinants recognized by a polyclonal antiserum. Cross-linking studies show that in each antigen the subunits are noncovalently associated in $\alpha_1\beta_1$ structures. Mac-1 and LFA-1 comprise a novel family of two-chain leukocyte differentiation antigens.

The distinct cellular distributions of the Mac-1 and LFA-1 antigens are emphasized by their reciprocal expression on



Fluorescence intensity (GF SRBC units)

Fig. 1 Reciprocal immunofluorescent labelling of peritoneal exudate cell macrophages and spleen cells by M1/70 and M7/14 MAb. Cells (50µl, 5×10⁷ ml in RPMI-1640, 5% fetal calf serum, 20 mM HEPES, 0.01 M NaN₃) were labelled with an equal volume of either M1/70 (a, b) or M7/14 (c, d) MAb-containing culture supernatants (dark dots) or NSI culture supernatant containing 50 μ g ml⁻¹ normal rat IgG as control (dim dots), washed, then labelled with affinity-purified fluorescein isothiocyanate rabbit F(ab')2 anti-rat IgG absorbed with mouse IgG. Immunofluorescence flow cytometry was performed on a Becton Dickinson FACS II equipped with a Nuclear Data log amplifier which was calibrated with glutaraldehyde-fixed sheep red blood cells (GF SRBC). Four-day thioglycollate-induced PEC (a, c) or spleen cells (d) were scatter gated to exclude red cells and lymphocytes, or red cells, respectively.

spleen cells and thioglycollate-induced peritoneal exudate cell macrophages (PEC) (Fig. 1). Spleen cells are >93% LFA-1 positive but only 6% Mac-1 positive, while exudate macrophages showed no significant LFA-1 expression but are >94% Mac-1 positive. Expression of Mac-1 and LFA-1 on 50 and 75% of bone marrow cells, respectively1,6, shows these antigens also have distinct myeloid distributions, and are co-expressed on a subpopulation of these cells.

Immunoprecipitation also confirmed that Mac-1 and LFA-1 are reciprocally expressed on concanavalin A-stimulated spleen

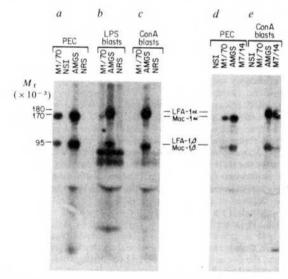


Fig. 2 SDS-PAGE of antigens precipitated from different cellular sources by M1/70 and M7/14 MAb and a polyclonal antiserum. Four-day Con A blasts (c, e), 4-day thioglycollate-induced PEC (>90% macrophages) (a, d) and LPS blasts (b) were prepared, surface labelled with 125 I, detergent solubilized, precleared, immunoprecipitated and subjected to SDS 5-15% gradient PAGE and autoradiography as previously described1,6. Two separate experiments (a-c and d, e) with independent cell preparations and SDS-PAGE were carried out. Cell lysates were immunoprecipitated with 25 or 50 μ l of NSI culture supernatant containing 50 μ g ml⁻¹ normal rat IgG (NSI) or 1 μ l of normal rat serum (NRS) as controls, 1 μ l of AMGS, 25 or 60 μl of M1/70 MAb supernatant (M1/70), or 20 μl of 1 mg ml⁻¹ M7/14 IgG (M7/14).

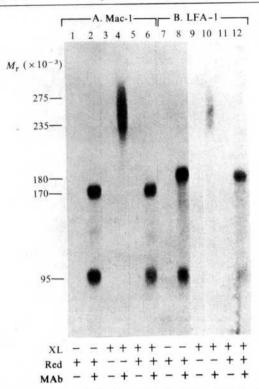


Fig. 3 Cross-linking of Mac-1 and LFA-1. PEC and EL-4 cells were surface labelled with ¹²⁵I using chloroglycoluril, and Triton X-100 cell lysates were prepared as previously described6, except haemoglobin carrier protein was omitted. Lysates were dialysed against 0.1 M NaCl, 0.05 M sodium borate, pH 9, and 800-μl aliquots were either mixed with 16 μl of dithiobis(succinimidyl propionate) (Pierce) dissolved in dimethylformamide, or not treated. After 1 h at 21 °C, samples were dialysed against two changes of 0.14 M NaCl, 0.05% NaN₃, 0.01 M Tris HCl pH 7.5. Aliquots were immunoprecipitated with antibodies coupled to Sepharose and subjected to SDS-5% PAGE and autoradiography as previously described6. a, precipitates from PEC with M1/70 MAb- or normal rat IgG-Sepharose. b, Precipitates from EL-4 with M17/4 MAb- (anti-LFA-1, Sanchez-Madrid et al., unpublished) or normal rat IgG-Sepharose. Samples were cross-linked (XL) and either reduced (Red) with 5% 2-mercaptoethanol or treated with 50 mM iodoacetamide before electrophoresis as indicated in the figure. Molecular weights were determined with standards as previously described6. Molecular weights >200,000 were determined by extrapolation and should be considered tentative. Crosslinked products were fuzzier than other bands, because the number and position of the cross-links formed affects hydrodynamic properties in SDS-

cells (Con A blasts) and PEC, and that the MAb do not cross-react between them. Thus, M1/70 but not M7/14 precipitated material from PEC (Fig. 2d), while M7/14 but not M1/70 precipitated material from Con A blasts (Fig. 2e). No cross-reaction between M1/70 and M7/14 could be detected, even when autoradiography was prolonged by a factor of five. This was as expected from the differing specificity of the MAb for cells (Fig. 1).

Despite these differences in cellular distribution and antigenic determinants recognized by MAb, Mac-1 and LFA-1 have strikingly similar two-chain structures. The LFA-1 α -chain is slightly higher in M_r than the Mac-1 α -chain, while the β -chains are identical in M_r (Fig. 2d, e). Determination of M_r by coelectrophoresis with standards in nongradient SDS gels at three different polyacrylamide percentages showed that the Mac-1 and LFA-1 α -chains are 170,000 and 180,000 M_r , respectively, and the β -chains 95,000 M_r (data not shown). The α - and β -chains are not disulphide linked^{1,6}.

Precipitation by MAb of two distinct polypeptide chains could be because both chains express the antigenic determinant, or because only one chain expresses the determinant but is non-covalently associated with the other chain. To investigate the quaternary structure of Mac-1 and LFA-1, cross-linking experiments were carried out. Detergent-solubilized lysates were cross-linked with the cleavable reagent dithiobis(suc-

cinimidyl propionate) and subjected to immunoprecipitation and SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 3). Instead of α - and β -chains (Fig. 3, lane 2), a cross-linked Mac-1 product of M_r 235,000–275,000 was found (Fig. 3, lane 4), in good agreement with the M_r of 265,000 predicted for an $\alpha_1\beta_1$ structure. Furthermore, the product was composed of α - and β -chains, as shown by reductive cleavage of the cross-links (Fig. 3, lane 6). A similar cross-linked product was obtained with LFA-1 (Fig. 3, lane 10). These results strongly suggest that both Mac-1 and LFA-1 contain α - and β -subunits which are noncovalently associated into $\alpha_1\beta_1$ quaternary structures.

One method of testing for structural homology between two proteins is by immunological cross-reactivity. MAb are not necessarily suitable, as they may recognize unique rather than shared antigenic determinants. Therefore, cross-reactivity was tested with a classical antiserum. A rat antiserum was raised against Mac-1 which had been partially purified from thioglycollate-induced macrophage membranes by L. culinaris lectin affinity chromatography and by MAb immunoadsorbent depletion of two other immunodominant antigens7. This antimacrophage glycoprotein serum (AMGS) potently precipitated Mac-1 and little or nothing else from PEC (Fig. 2a, d). The AMGS also precipitated large amounts of material from lipopolysaccharide-stimulated spleen cells (LPS blasts) and Con A blasts with an Mr identical to that of LFA-1, while this material was not precipitated by M1/70 (Fig. 2b, c). The material precipitated by AmGS from PEC and Con A blasts was shown to be identical to Mac-1 and LFA-1, respectively, by co-migration in SDS-PAGE (Fig. 2d, e), and by Cleveland peptide mapping (data not shown).

To determine whether precipitation of LFA-1 by AMGS was due to a true cross-reaction of anti-Mac-1 antibodies with LFA-1, or to the presence of antibodies with independent specificities in the AMGS, it was important to test whether the anti-LFA-1 activity could be absorbed with thioglycollate-elicited macrophages, which are Mac-1⁺ LFA-1⁻. Therefore, AMGS was absorbed with either macrophages or Con A blasts, and the amount of LFA-1 precipitated from Con A blasts was measured by quantitative scanning of autoradiograms (Fig. 4). Despite their lack of LFA-1 expression, macrophages gave

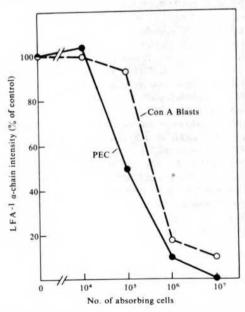
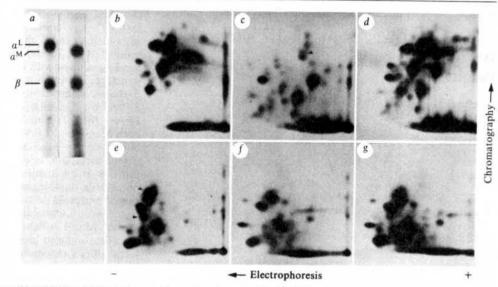


Fig. 4 Absorption by peritoneal exudate macrophages and Con A blasts of the ability of AMGS to precipitate LFA-1 from Con A blasts. AMGS (10 μl of a 1:100 dilution, which gave 70% maximal LFA-1 precipitation) was absorbed with PEC or Con A blasts in 100 μl of phosphate-buffered saline for 2 h at 4 °C. Cells were pelleted, and 80 μl of supernatant was used in indirect precipitation of 125 I-labelled Con A blast lysates and subjected to SDS-PAGE as described in Fig. 2 legend. After autoradiography with intensifying screens and hypersensitized Kodak XAR film, the film was scanned with a densitometer and LFA-1 α-chain was quantified as peak

Fig. 5 Tryptic peptide maps of SDS-PAGE purified LFA-1 and Mac-1 α - and β-subunits. LFA-1 and Mac-1 were purified from EL-4 and P388D, cells, as will be described in detail elsewhere. Briefly, EL-4 or P388D, detergent lysates in the presence of protease inhibitors were purified by affinity chromatography on M7/14 or M1/70 coupled to Sepharose CL-4B, respectively, and eluted with 20 mM glycine-NaOH, pH 10, or 20 mM triethanolamine, pH 11, respectively, in 0.5% Triton X-100, 1 mM phenylmethylsulphonyl fluoride. Eluates were neutralized and repurified by a second cycle of affinity chromatography. Purified antigens were concentrated and dialysed against phosphate-buffered saline, and 10-15 μg was iodinated in a volume of 100 μl with 1 mCi ¹²⁵I and 6 μg chloramine-T (ref. 10) for 1 min, followed by addition in sequence of $6 \mu l$ of $1 \text{ mg ml}^{-1} \text{ Na}_2 S_2 O_5$, $5 \mu l$ of 20 mg ml^{-1} KI and 5 μl of 20 mg ml⁻¹ bovine serum albumin. Rat IgG was also iodinated and treated in parallel as a control. After



dialysis, the α and β polypeptides were separated by SDS-7% PAGE and the wet gel was autoradiographed for 10 min (panel a). a, Lane 1, LFA-1; lane 2, Mac-1. dialysis, the α and β polypeptides were separated by SDS-1% PAGE and the wet get was autoradiographed for 10 min (panel a). a, Lane 1, LFA-1; lane 2, Mac-1. Bands were excised and digested with TPCK-trypsin as described by Elder et al. Peptide aliquots (5–8×10⁴ c.p.m. in 2–4 μ l) were spotted on 10×11.5 cm cellulose TLC plates (EM Laboratories). Electrophoresis was in 15% acetic acid, 5% formic acid, chromatography was in n-butanol/pyridine/acetic acid/water(65:50:10:40), followed by autoradiography. Comparison to the control rat IgG peptides showed that none of the spots were due to free 125 I or simple adducts. b, LFA-1 α-chain; c, Mac-1 α-chain; d, mixture of α-chains; e, LFa-1 β-chain; f, Mac-1 β-chain; g, mixture of β-chains. The arrows in b, c and d are explained in the text.

complete absorption. Furthermore, the macrophages were three-fold more effective than Con A blasts in absorbing anti-LFA-1 antibodies, showing absorption could not be attributed to contaminating lymphoid cells. The greater effectiveness of the macrophages was consistent with our previous findings that macrophages express 16×104 Mac-1 sites per cell while Con A blasts express 7.8 × 10⁴ LFA-1 sites per cell^{2.6}. It was concluded that antibodies to Mac-1 cross-react with LFA-1, and hence these molecules are structurally related.

The structural basis for this homology between Mac-1 and LFA-1 was investigated by peptide mapping. Mac-1 and LFA-1 were purified from the P388D1 macrophage tumour line and the EL-4 T-lymphoma line, respectively, by MAb affinity chromatography, and labelled with ^{125}I . The α - and β -subunits were separated by SDS-PAGE (Fig. 5a), excised from gels and subjected to peptide mapping. Tryptic peptide mapping showed the Mac-1 and LFA-1 β-subunits share at least 10 tyrosyl peptides, and are thus highly homologous or identical (Fig. 5e-g). One peptide unique to the LFA-1 β -subunit and one increased in intensity (arrows) may be due to carbohydrate processing differences between the EL-4 and P388D1 tumour lines, rather than to sequence differences. Cleveland peptide maps of LFA-1 and Mac-1 β -chains isolated from Con A blasts and PEC were identical.

The α -subunits had very different tyrosyl tryptic peptide maps (Fig. 5b-d). The α -chains from Mac-1 and LFA-1 showed 17 and 18 unique tryptic peptides, respectively. Co-migration of one peptide (marked by an arrow) was confirmed by a mixing experiment (Fig. 5d). These extensive differences strongly suggest that the Mac-1 and LFA-2 α-chains are products of distinct genes.

The large structural differences in the α -subunits suggest that the unique determinants recognized by the M1/70 and M7/14 MAb reside on these subunits. The β -subunits seem to be immunoprecipitated by virtue of their noncovalent association with the α -subunits. The α - and β -subunits do not appear structurally related, because they showed no tryptic peptide map identities. It seems likely that the common determinants on LFA-1 and Mac-1 revealed by polyclonal anti-Mac-1 antibodies are on the β -chain, although it is possible that the α -chains also cross-react. Definitive proof of where the determinants lie will require testing of antibodies on isolated chains.

Mac-1 and LFA-1 comprise a novel family of leukocyte differentiation antigens which contain noncovalently associated

a and β polypeptides of 170,000-180,000 M_r and 95,000 M_r . respectively. Alternative forms of the α polypeptide can be associated with a common or highly homologous β polypeptide in $\alpha_1\beta_1$ structures. Only two other two-chain leukocyte cellsurface antigen families have previously been described, the major histocompatibility antigens8 and immunoglobulins9. By analogy with these and other protein families such as the haemoglobins which have shared homologous or identical subunits, it seems likely that the Mac-1 and LFA-1 α -chains are also products of closely linked, homologous genes. The tryptic peptide differences in the α -subunits do not rule out homology, since amino acid sequence homologies of the order of 25-50% would be missed by peptide mapping. The selective expression of the Mac-1 and LFA-1 α-chains in the monocytic and lymphoid lineages is a particularly interesting feature of this differentiation antigen family. The structural relationships between these molecules suggest that the α - and β -chains may mediate specialized and general functions, respectively. LFA-1 plays an important part in antigen-specific T-lymphocytemediated killing of tumour cells and in T-helper cell responses4.5. Elucidation of the function of Mac-1 would provide a further comparison with LFA-1, and might provide important insights into the structure-function relationships of the α - and B-subunits.

We thank E. Minicucci for assistance in manuscript preparation. This research was supported by NIH grants CA 31798 and CA 31799.

Received 20 October 1981; accepted 19 February 1982.

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Enhanced transformation of human fibroblasts by origin-defective simian virus 40

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Transformation of semipermissive human fibroblasts (HF) by wild-type simian virus 40 (SV40) or SV40 DNA is relatively inefficient compared with SV40 transformation of non-permissive rodent cells1. Whereas HF transformed with either SV40 or a subgenomic fragment of SV40 (that is incapable of making virions) containing the early region and the origin of DNA replication produce large amounts of free virus DNA2 lished human cell lines transformed by SV40 harbour defective virus genomes6 that are incapable of initiating virus DNA replication (M. Botchan, personal communication). We have now investigated whether the low efficiency of transformation is directly related to the ability of SV40 DNA to replicate autonomously in semipermissive HF. We have compared the efficiency of transformation of HF by origin-defective SV40 DNA (SV ori-) with that of other derivatives containing a functional viral origin of replication, using the calcium phosphate co-precipitation technique⁷. The transforming potential of SV ori mutants was found to be superior.

The SV ori mutant is a hybrid DNA consisting of plasmid (pMK16) and the full genome of SV40 DNA less 6 nucleotides at the BgII site^{8,9}. It is able to transform rat cells as efficiently as the ori SV40 derivative, but, unlike the latter, is able to transform permissive monkey cells¹⁰. The cell line chosen for the present studies was a human fibroblast designated HS74BM' (HF), which is derived from fetal bone marrow and displays a diploid human karyotype¹¹. This cell line is semipermissive for SV40, producing virus at an efficiency of ~1% that of permissive monkey kidney fibroblasts¹². It has been shown to be transformable by DNA transfection with an early region fragment of SV40, albeit at a low frequency⁵.

Initial experiments revealed that the SV ori recombinant molecule was capable of transforming HS74 as assayed by focus formation in monolayer and colony formation in soft agarose ('anchorage independence'). Transformed colonies express T antigen as detected by immunofluorescence. The actin cable organization of these transformants was consistent with typical SV40 transformants (R. Pollack, personal communication).

Direct quantitative comparison among intact superhelical form I SV40 DNA, plasmid SV ori⁺, plasmid SV ori⁻, as well as among linear form III plasmid SV ori⁺ and plasmid SV ori⁻ is shown in Table 1. Figure 1 shows typical foci. pSV ori⁻ DNA was most efficient in both transformation assays (focus formation and anchorage independence) as well as in the different protocols used after DNA transfection (see Table 1 legend). The results are most striking for the anchorage independence assay, which is considered the more stringent *in vitro* transformation assay¹³. It is also the most stringent for cell viability because it requires colony formation.

This finding strongly suggested that the absence of a functional origin of replication in SV ori⁻ resulted in its enhanced transformation efficiency. However, as plasmid SV ori⁺ DNA had a higher transforming potential than did virion DNA, other possibilities were considered. First, the improved efficiency might be solely due to the presence of adjoining plasmid sequences and/or modification of DNA through propagation in a bacterial host¹⁴. As shown in Table 2, we compared the efficiency of transformation by linearized virion DNA with linear viral DNAs derived from pSV ori⁺ and pSV ori⁻, from which plasmid DNA was removed (lines 1, 3, 4). Again SV ori⁻ was the most efficient. Residual contribution from the bacterial

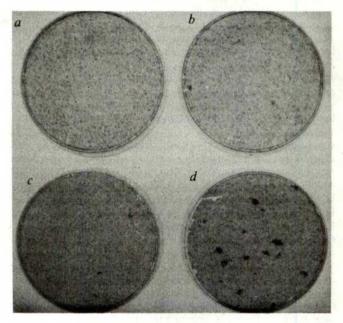


Fig. 1 Giemsa-stained plates of HS74BM 3 weeks post-transfection with SV40 DNA (form I) as described in Table 1 legend. a, no SV40 DNA; b, SV40 virion DNA; c, pSV ori DNA; d, pSV ori DNA.

Table 1 Transformation of HF by form I and form III DNAs

	In situ	3 Day su	bcultivation	7 Days	ubcultivation
DNA derivative	foci*	Foci	Colonies in agarose	Foci	Colonies in agarose
SV40 Virion I	0.5(0, 1)	0.7 (0, 1, 0, 2)	0.5(0, 1, 0, 1)	6.0 (4, 8)	0.0(0,0)
pSV ori+ I	1.5 2, 1)	2.0 (2, 0, 2, 4)	2.7 (6, 0, 2)	1.5(2, 1)	0.5(0, 1)
pSV ori- I	3.5(2,5)	8.5 (5,14, 10, 5)	3.3 (2, 2, 6)	10 (10, 10)	6.0(2, 10)
pSV ori+ III	8.0 (7, 9)	12 (16, 10, 10, 12)	1.0 (3, 0, 1, 0)	14 (14)	0.5(0, 1)
pSV ori III	25 (25)	31 (31, 28, 35, 32)	20 (19, 27, 22, 13)	60 (60)	18 (16, 20)
Salmon sperm	0.0(0,0)	0.0(0,0,0,0)	0.0 (0, 0, 0, 0)	0.0(0,0)	0.0(0,0)

SV40 virion form I DNA was extracted from SV40-infected monkey kidney cells (CV-1) by the technique of Hirt¹⁸ and isolated by CsCl-EtBr equilibrium centrifugation¹⁹. The DNA was further purified by deproteinization with redistilled phenol/CHCl₃/isoamyl alcohol, precipitated in 2 volumes 95% ethanol, redissolved in TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) and stored at 4 °C before use. pSV ori* and pSV ori¬ plasmid DNAs were isolated from Escherichia coli χ1776 by the clear lysate technique²⁰ and purified as described above. Form III derivatives were prepared by digestion of form I with XhoI (which makes a single cut within pMK16); DNA was deproteinized and reisolated after ethanol precipitation. HS74 were utilized at the eighth passage. Culture medium was a 1:1 mixture of Dulbecco's modified Eagles medium (DMEM) and Ham's F-10 (MABioproducts) supplemented with 15% fetal calf serum ("complete media"), unless otherwise noted. Cells were grown in a 5% CO₂ atmosphere at 37 °C. DNA transfections were performed essentially as described by Wigler et al.²¹: 20 h before transfection, cells were plated at 7.5 × 10⁵ per 60 mm Petri dish. Cultures were refed 4 h before transfection with 4.5 ml of DMEM supplemented with 5% calf serum. DNA-calcium phosphate precipitates were prepared such that each dish received 0.5 ml containing 0.5 μg SV40 genome equivalents and 20 μg high molecular weight salmon sperm DNA (as carrier). Cells were exposed to DNA for 4 h, then refed with complete medium. Cultures were incubated for 3 weeks and scored for transformed foci (in situ selection). In the subculture protocol, cultures were trypsinized 3 and 7 days post-transfection; one-half of each dish was reseeded into a 100 mm Petri dish and scored 3 weeks later for focus formation; the other half was suspended in 0.35% agarose⁵, and scored after 3 weeks for macroscopic colonies 0.1 mm in diameter).

* Data presented as average number of foci or colonies per plate, with individual points in parentheses.

Table 2 Transformation of HF by form III DNAs

			2		
DNA derivative	In situ		bcultivation	7 Day s	subcultivation
SV40 virion III SV40 virion Eco/Bam SV ori ⁺ III SV ori ⁻ III	foci 2.5 (1, 4) 1.5 (1, 2) 4.5 (1, 8) 9.5 (8, 11)	Foci 3.3 (3, 5, 2, 3) 2.5 (2, 3, 2, 3) 6.7 (4, 7, 9) 8.3 (10, 5, 8, 10)	Colonies in agarose 0.3 (0, 1, 0) 0.5 (1, 0, 1, 0) 2.0 (4, 0, 2, 2) 4.3 (5, 6, 3, 3)	Foci 7.0 (1, 13) 0.0 (0, 0) 9.0 (9) 25 (25)	Colonies in agarose 0.0 (0, 0) 0.0 (0, 0) 1.5 (0, 3) 20 (19, 22)

SV40 virion form I DNA was extracted from SV40-infected monkey kidney cells as described in Table 1 legend. Virion form III DNA was generated by digestion of form I DNA with BamHI. The subgenomic Eco/Bam fragment was isolated by simultaneous digestion of virion form I DNA with EcoRI and BamHI; the DNA was then electrophoresed on a 1% preparative agarose gel in Tris-borate buffer at 25 V for 18 h. DNA was visualized under UV light by ethicium bromide staining; and the 4,670 base pair fragment was cut out from the gel and reisolated by electroelution²². pSV ori and pSV ori plasmids were isolated as described in Table 1 legend. SV ori and SV ori form III DNAs (viral sequences from which plasmid sequences were removed) were generated by digestion of form I plasmid DNA with BamHI, which separates SV40 from pMK16 sequences; viral DNA was isolated by agarose electrophoresis and electroelution as above. Cell culture, DNA transfections and all transformation assays were performed as described in Table 1 legend. Restriction enzyme digests were performed according to suppliers' (New England Biolabs and Bethesda Research Labs) specifications.

environment (such as methylation) appears likely as well because SV ori+ form III transforms with higher efficiency than viral DNA isolated from permissive monkey cells. Note, however, that the contribution of bacterial modification seems to be transient, and almost completely disappears after 7 days (see Tables 1, 2). Second, we tested whether secondary infection was responsible for the reduced transformation efficiency of viral DNA by using a viral DNA molecule which had been deleted of 750 base pairs in the late region, precluding formation of viral particles 15,16. No improvement was observed (compare lines 1 and 2 in Table 2).

These experiments demonstrate that the efficiency of transformation of semipermissive human fibroblasts can be markedly increased by using a viral DNA which is defective at the origin of DNA replication. Transformation with SV40 ori- mutants resulted in an increased number of transformants as observed by both direct assay (for example, in situ) and subculture assays for anchorage independence. Furthermore, foci and agarose colonies generated with pSV ori DNA were larger than those generated by SV40 derivatives containing a functional viral origin of replication (see Fig. 1). The transformation frequency can be further increased by using linearized pSV ori DNA which gives markedly better results than conventional viral DNA I. The frequency of transformation of HF by pSV ori DNA increases as a function of the amount of DNA used in transfection. However, this increase is non-linear, and approximating threefold over the range 1-10 µg per culture. Increasing the amount of SV40 virion DNA in the same manner does not substantially influence the transformation frequency (data not shown). We are now investigating whether HF transformed by SV ori DNA are more likely to become permanently established¹⁷. Preliminary Southern blot analysis of the Hirt (low molecular weight DNA) supernatants of transformants generated by various SV40 derivatives has failed to detect production of free SV40 viral DNA in SV ori -- derived transformants, as predicted (D. Neufeld and H. Ozer, unpublished results).

In conclusion, we believe that the origin-defective recombinant SV40 molecule is the vector of choice for SV40 transformation of human fibroblasts. Its enhanced transformation efficiency (relative to wild-type SV40) is probably due to its inability to excise and replicate in transformed semipermissive cells. This property should increase the stability of the transformed phenotype. Furthermore, it precludes the possibility of cell death mediated by uncontrolled viral DNA synthesis or production of virus particles. We observe that SV ori DNA linearized in its plasmid sequence by digestion with the restriction enzyme XhoI routinely gives the highest transformation frequencies (~1 in 105 or greater can be obtained on transfection of early passage cells). Transformants of human fibroblasts generated by this vector may prove particularly useful in the establishment of human cell lines for various purposes.

We thank Ms Ruth Kalmanowitz for technical assistance, Dr Krishna K. Jha for helpful discussions, and Drs Richard Frisque and James Stringer for critical reading of the manuscript. This work was supported by NIH grants CA 23002 and CA 23003 (H.L.O.) and CA 13106 (Y.G.). The HS74BM cells were provided by the Cell Culture Laboratory, Naval Biochemical Research Laboratory, Oakland, California, under contract E-73-2001-NO1 within the NIH Special Virus-Cancer Program.

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Enhanced metastasis of tumours induced by a SV40 small T deletion mutant

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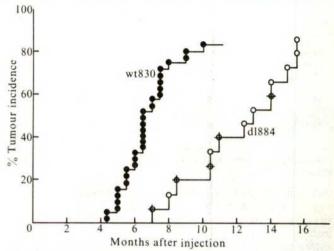
The lethality of tumours is determined largely by their ability to metastasize to distant sites. Once metastasis has occurred, eradication of the tumour cells from the body becomes difficult or impossible. Although the steps involved in tumour metastasis have been well documented (for review see refs 1-3), the cellular parameters that influence the tendency of tumours to metastasize have been more difficult to define 4-7, and the underlying genetic determinants of metastatic potential are not known. We are studying the metastasis of tumours induced in hamsters by simian virus 40 (SV40). We show here that tumours induced by a viral mutant (d1884) in the small T antigen gene tend to metastasize, whereas tumours induced by wild-type SV40 rarely do so. Thus, we have a well defined system in which the underlying genetic differences in the transforming viruses are known and in which we may be able to correlate these differences with cellular parameters that determine tumour metastasis.

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Fig. 1 Time course of tumour induction by wild-type SV40 (wt830) and the small T deletion mutant (d1884). Newborn <24 h of age) Golden Syrian hamsters (strain LVG, Charles River) were injected subcutaneously between the shoulder blades with $\sim 1 \times 10^8$ plaque-forming units (PFU) of either viral strain. (The small T deletion mutant d1884, constructed from the parental strain wt830 by Shenk *et al.*¹⁶, eliminates 253 base pairs¹⁷ in the 0.54-0.59 map unit segment of the early region of SV40. Thus, the coding sequence for the C-terminal 63 amino acids of the 174 amino acid small T protein are deleted. The coding sequences for the large T protein remain intact.) Animals were palpated for tumours at least once per week and the age of the animal at the time the tumour was first observed was recorded. The 'latency period', or the time required for the tumour to become observable, is plotted on the abscissa. Thirty animals were in the group injected with wild-type virus and 15 were in the group injected with the mutant virus. Animals displaying metastases are indicated by the crossed symbols.

We first compared the ability of wild-type SV40 (wt830) and the small T deletion mutant (d1884) to induce tumours following subcutaneous injection in newborn hamsters. The d1884 mutant is deficient in transformation of rodent cells in culture in some conditions⁸⁻¹⁰ and it was shown to cause tumours in animals at reduced frequency^{11,12}. We found that d1884 induces tumours at a similar frequency to wild-type SV40 but that these tumours appear after a longer latency period (Fig. 1)—a median latency period of 12.5 months, compared with 6.5 months for wild-type virus. The most striking finding, however, was that some of the animals injected with the d1884 virus (indicated by crosses in Fig. 1) developed tumour foci at sites distant from



the subcutaneous injection site. In some cases tumour foci were found only on internal organs or in the abdominal cavity, whereas in others both subcutaneous tumours and distant tumour foci were present (see Table 1). In all cases histological examination showed the neoplastic cells in the tumours and organs to be fibrosarcomas.

Of 36 animals injected with wt830, 30 animals developed tumours. The tissues of 12 animals with wild type-induced tumours were examined macroscopically and histologically and no abdominal tumours or metastases were observed.

To prove that the subcutaneous tumours and distant tumour foci actually arose from d1884-transformed cells, we have

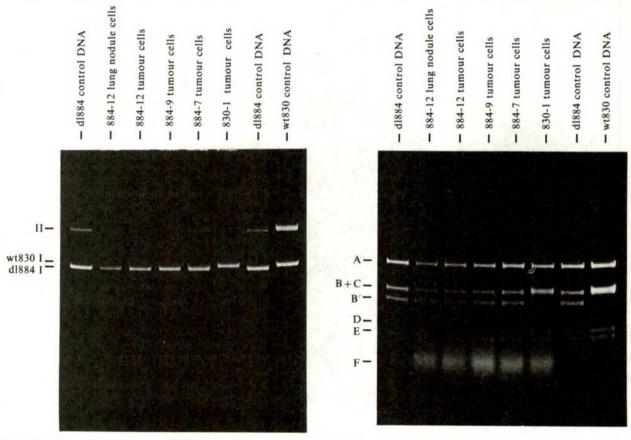


Fig. 2 Agarose gel electrophoresis of DNA from virus rescued from SV40-induced tumours. Tumours were removed from the animals and cell lines established by disaggregation of tumour pieces with collagenase and culture in minimal essential Eagle's medium with Earles salts, 5% fetal calf serum and 5% calf serum. Virus was rescued from these cell lines by culturing them together with permissive CV-1 cells (African green monkey kidney line CV-1 clone TC 7; ref. 18). Virus was collected after 14 days and used to infect more CV-1 cells. After 3 days the viral DNA was extracted from these cells by the method of Hirt¹⁹. The extracted DNA was electrophoresed on 0.8% agarose gels or cleaved with HindIII (New England Biolabs) and then electrophoresed. The direction of migration is from top to bottom in the photograph. Left panel, uncleaved viral DNA; right panel, viral DNA cleaved with HindIII. The numbers above designate individual animals. Roman numerals to the side refer to SV40 form I and II molecules. Letters to the side refer to the HindIII restriction fragments. The mutant d1884 deletion is within fragment B.

Table 1 Characteristics of hamsters exhibiting metastases

Animal	Site of principle tumour	Time at which hamster died or was killed	Location of metastases
884-1 884-2 884-5 884-6 884-9	None Abdominal None Abdominal Subcutaneous, abdominal	Died at 8.5 months Died at 7 months Died at 10.5 months Died at 11 months Killed at 14 months (14 days after subcutaneous tumour was observed)	Lung, spleen Peritoneum, lung Liver, pancreas, colon and lymph node Spleen, liver and intestine Peritoneum, diaphragm, liver
004-12	Subcutaneous	Killed at 11 months (45 days after subcutaneous tumour was observed)	Pleural surface

Animals were necropsied as soon as possible after death or immediately after killing. Gross observations were made and tissues prepared for histology.

characterized virus rescued from cultured tumour cells by cocultivation with permissive monkey cells (CV-1 line). Rescued virus was used to infect CV-1 cells and viral DNA was prepared from a Hirt supernatant fraction. Cleaved (*HindIII*) and uncleaved viral DNA was electrophoresed on 1% agarose gels, stained with ethidium bromide and photographed (Fig. 2). Viral DNA rescued from d1884-induced tumour cells co-electrophoreses with d1884 viral DNA; viral DNA rescued from wt830-induced tumour cells co-electrophoreses with wt830 viral DNA. Note that d1884 viral DNA was rescued from cells recovered from a metastatic lung nodule as well as from the subcutaneous primary tumour.

The distant tumour foci that we observed arose either as metastases from a primary tumour or by independent virusmediated transforming events. These two possibilities may be distinguished by comparing the viral genome integration patterns in cells from the primary tumours with those in cells from the distant tumour foci. If the distant tumour foci arose by independent transforming events, the viral genome integration patterns would be likely to differ from those of the primary tumour, because SV40 viral DNA seems to integrate at random^{13,14}. If these distant tumour foci are metastases from the primary tumour, their viral integration patterns should be the same.

We analysed the viral genome integration patterns from primary tumours and metastases. Cellular DNA was extracted from cultured tumour cells, treated with restriction endonucleases, electrophoresed on agarose gels, transferred to nitrocellulose sheets¹³ and hybridized with the ³²P-labelled nick-translated SV40 DNA probe¹⁴. Analysis of the integration patterns in cultured cells from several of the primary subcutaneous tumours, using restriction enzymes that cut within the SV40

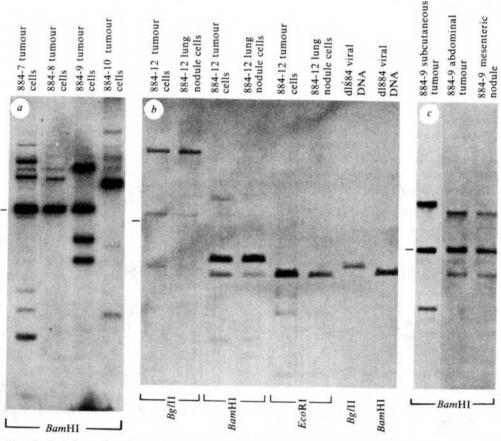


Fig. 3 Hybridization of restriction endonuclease-cleaved cellular DNA from small T deletion mutant-induced tumours and metastases with the SV40 probe. Cellular DNA was extracted from cultured tumour cells by the method of Botchan et al.²⁰ or from frozen tumour pieces as described by Blin and Stafford²¹. DNA was then cleaved with BamHI, Bg/II or EcoRI (New England Biolabs) and electrophoresed on a 0.8% agrose gel. DNA was transferred to nitrocellulose (as described by Botchan et al.¹³) and hybridized with ³²P-labelled nick-translated SV40 DNA (~1×10⁸ c.p.m. per μg) according to the method of Wahl et al.¹⁴. The position of the unit length linear SV40 band is indicated by the dashes in the figure. a, Cellular DNA was prepared from cultured cells derived from primary tumours of four different animals. b, Cellular DNA was prepared from cultured cells derived from a primary tumour and a lung nodule from the same animal. (The Bg/II enzyme preparation contains a nicking activity that converts SV40 form I DNA to form II.) c, Cellular DNA was extracted from frozen tumour pieces of three separate tumours present in a single animal.

Table 2 Metastasis of re-injected tumour cells

Tumour cells	No. of cells inoculated	Tumour incidence	Tumour latency [median (range), in days]	Metastasis incidence
884-12 884-12 830-1 830-1	3×10^{4} 3×10^{3} 3×10^{4} 3×10^{3}	4/4 5/5 5/5 3/5	25(25–30) 30(25–37) 30(25–92) 81(49–91)	4/4 5/5 1/5 0/5

Tumour cells were passed in culture, then trypsinized and injected subcutaneously into 7-week old hamsters. Animals were killed and examined for metastases ~42 days after the subcutaneous tumours were first observed.

genome either once or not at all, indicated that the integration patterns are relatively simple (only a few integration sites) and that there is a small amount of free viral DNA. Furthermore, we found a unique pattern in each of the cell lines derived from subcutaneous tumours in different animals (Fig. 3a). Most importantly, we found that the integration pattern in cells from a primary tumour and lung nodules from the same animal are identical (Fig. 3b). Thus, we conclude that the cells in the lung tumour nodules are actually derived by metastasis from the subcutaneous primary tumour. However, not all of the distant tumour foci seem to be derived as metastases from a subcutaneous tumour. For example, one animal had a subcutaneous tumour and an abdominal tumour exhibiting distinct viral integration patterns (Fig. 3c). Additional small abdominal tumour foci had patterns identical to the major abdominal tumour (Fig. 3c). Thus, either the abdominal tumours were derived as metastases from an undetected subpopulation of cells in the subcutaneous tumour or they arose from an independent transforming event perhaps occurring in the abdomen. We never observed abdominal tumours following subcutaneous injection of the wild-type virus. If the development of the abdominal tumours is unrelated to metastasis, it could be due to a change in the cell specificity of the virus particle itself.

What causes the difference in metastasis of tumours induced by wild-type SV40 and the small T deletion mutant? We believe that the observed metastasis is not a result of the increased latency period exhibited by the small T deletion mutant. Metastatic tumours arose after latency periods similar to non-metastatic tumours observed in many of the animals injected with the wild-type virus. Furthermore, it is unlikely that the metastasis is due to a prolonged presence of the primary tumour in the animal. Deichman and Kluchareva¹⁵ showed that metastasis of wild type-induced tumours sometimes occurred when tumours were allowed to develop for more than 60 days after they had first appeared; in our experiments, animals were usually killed at much earlier times after the tumours first appeared because later on the tumours become quite large and the animals appeared sickly. Instead, the tumour metastasis seems to be related to properties of the tumour cells themselves. We have determined that cells derived from metastatic tumours and passed in culture retain their ability to metastasize on reinjection in adult animals; cells from wild type-induced tumours metastasize less readily in these conditions (Table 2). Topp et al. 12 compared cells derived from wild type- and small T mutant-induced tumours for certain transformed properties (the ability to grow suspended in methylcellulose, loss of actincontaining cable networks and synthesis of plasminogen activator). Although no differences between the wild type- and mutant-induced tumour cells were observed, metastasis was not monitored in their studies, and it is unclear whether any of the cell lines analysed were derived from metastatic tumours.

Further analysis of the properties of our tumour-derived cell lines may reveal characteristics which explain their difference in metastatic potential. Once these characteristics have been identified, we will be able to investigate how they are determined by the genetic make-up of the transforming viruses.

This research was supported by seed money research grants from California Institute for Cancer Research and Cancer Research Coordinating Committee. B.-J.R. was supported by USPHS National Research Service Award GM07185. A donation of funds from the Rita Burro Fund is gratefully acknowledged. We thank Dr. Helgard Niewisch for evaluation of histological sections and advice about animal care, and Dr Daniel W. Nebert for helpful discussions and critical reading of the manuscript.

Received 9 November 1981; accepted 1 February 1982.

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Activation of non-expressed bovine papilloma virus genomes by tumour promoters

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Naturally occurring cancer seems to be a multiple-hit phenomenon resulting from the interaction of several factors. Numerous environmental carcinogens on one hand and the widespread tumour viruses on the other, are capable of either transforming cells in vitro or inducing tumours in vivo. A few years ago, based on epidemiological evidence, a possible interaction between bovine papilloma viruses and an environmental carcinogen in bracken fern was noted in conjunction with alimentary cancer in cattle1. We report here that tumour-promoting agents which by themselves are unable to transform cells, are capable of inducing the transcription of bovine papilloma virus type 1 (BPV-1) in mouse embryo fibroblast (MEF) tissue cultures, where the viral genomes reside in a non-expressed episomal state after infection. In such cells, one brief treatment with the tumour promoter results in the transcription of the same viral mRNA species present in BPV-1-induced tumours and in BPV-1-transformed cells. Furthermore, viral DNA is replicated and the cells acquire the transformed phenotype. Once activated, these properties remain stable. This interaction between tumour promoters and latent inactive tumour virus genomes leads, in appropriate cell systems, to the activation of particular viral genes and to the transformed phenotype of the host cells.

BPV can induce fibroblastic tumours in animals²⁻⁴ and is able to transform tissue culture cells^{5,6}. Unlike most other tumour viruses, however, BPV achieves transformation as an unintegrated episome⁷⁻¹². A further peculiarity of BPV is the lack of a permissive tissue culture system. Virion production occurs exclusively in the differentiated periphery of the virus-induced warts¹³. Hence, as a rule, infection of tissue culture cells leads, if at all, to only a partial expression of the viral genome¹⁴ with ensuing transformation. Not all tissue isolates from one species are transformable; only 2 of 11 fetal bovine skin cultures could be transformed with BPV¹⁵. Similarly, not all mouse-derived

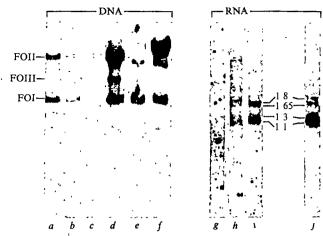


Fig. 1 Effect of TPA on BPV-1-infected MEF cells MEF cells (DBA strain, passage 5) were infected with a BPV-1 virus pool (0 5 ml per 10 which had been prepared as follows a bovine wart was minced in an Omnimix at 4 °C for 10 min (10% w/v in phosphate-buffered saline). After pelleting at 20,000g for 20 min, the virus-containing supernatant was sterilized by passage through a Millipore filter and used to infect MEF cells. The viral DNA showed a typical BPV-1 restriction enzyme cleavage pattern The infected cells were passaged once a week at a 1.5 split ratio in Eagle's basal medium supplemented with 10% fetal bovine serum DNA and RNA were prepared 4 days after plating when the cells had reached confluence, and were analysed by gel electrophoresis either in 1% or 14% agarose as described elsewhere²² After phenol extraction, the RNA was selectively After phenol extraction, the RNA was selectively precipitated with 2 M LiCl and traces of DNA were removed by treatment with DNase I (grade I) as detailed elsewhere Transfer to mitrocellulose filters, hybridization with a nick-translated ³²P-labelled BPV-1 DNA probe (cloned in pBR322 and provided by P Howley), and autoradiography were as described elsewhere 22 In each track of the gels 5 μ g of DNA and 20 μ g of RNA were assayed a-f, DNA from BPV-1-infected MEF cells a-c, 1, 2 and 3 passages, respectively, after infection, d, same as a, but exposed for 4 days to 10⁻⁷ M TPA; e, same as d, but in the first passage after removal of TPA, f, same as d, but in the fifth passage after removal of TPA. g-1, RNA from the same cultures as above. g, MEF cells 1 passage after infection; h, 1 passage after infection and exposure for 4 days to 10^{-7} M TPA, l, same as h, but in the second passage after removal of TPA; , RNA from BPV-1-transformed hamster embryo fibroblast cells in the 16th passage after infection. The values on the right indicate the sizes of the BPV-1 RNA sequences14 in kb

fibroblast tissue cultures could be transformed, for example, NIH 3T3 and C 127 responded to BPV-1 infection with transformation⁸, while repeated efforts in our laboratory to transform fibroblasts from the DBA strain were unsuccessful.

Following passaging of the BPV-1-infected phenotypically normal cells, the viral DNA is eventually lost because of its failure to replicate. This is shown in Fig. 1a–c, where the method of Southern¹⁶ was used to reveal BPV-1 DNA after infection of MEF cells with BPV-1 virions. One passage after infection, superhelical form I (FOI) and relaxed FOII BPV-1 DNA were detected in the cells using a 32 P-labelled, nick-translated BPV-1 DNA probe. After the second passage, most of the viral DNA had disappeared (Fig. 1b), and after a third passage no viral DNA was detectable (Fig. 1c). It has been shown that 12-O-tetradecanoylphorbol-13-acetate (TPA) has a stimulatory effect

on Epstein-Barr virus¹⁷ and episomal papovavirus genomes¹⁸ and this is confirmed in Fig. 1d. A parallel culture to that shown in Fig. 1a was subjected to TPA treatment and, when examined 4 days later displayed considerably more BPV-1 DNA, particularly FOII and some unit-length FOIII. This effect was observed as early as 18 h after treatment with TPA. Even when administered to cells in the third passage after infection, TPA amplified the minute amounts of persisting non-detectable BPV-1 DNA (see Fig. 1c) to the extent that a clearly visible signal was obtained by the Southern method (data not shown)

Furthermore, the BPV-1 DNA continues to replicate in the absence of TPA. Figure 1e, f show the viral DNA content at one and five passages after TPA removal. In addition to FOI and FOII, multimeric BPV-1 DNA structures are present. These car be converted to linear FOIII after cleavage with single-currestriction endonucleases (data not shown).

The effect of TPA on viral transcription is shown in denaturing gels (Fig. 1g-j)¹⁹. The total RNA was extracted from the same cultures used to analyse DNA. Although untreated MEI cultures contain BPV-1 DNA one passage after infection (Fig 1a), no viral RNA sequences could be detected (Fig. 1g) However, in a parallel culture, 4 days after TPA treatment (Fig 1h; see Fig. 1d for the DNA content), several species of BPV-RNA were found. The same RNA species are also found in the polyadenylated RNA of BPV-1-induced hamster tumours and BPV-1-transformed hamster cells¹⁴ (Fig. 1j). That these bands represent true RNA sequences is shown by their absence after RNase or alkali treatment (data not shown). Viral transcription persisted during subsequent passages in the absence of TPA (Fig. 1i). In agreement with our previous findings, the 2-kilobase (kb) mRNA which probably encodes the viral capsid protein and which is present exclusively in the permissive periphery of the warts¹⁴, was absent. The faint larger bands were observed only occasionally and may represent mRNA precursors.

Together with the induction of viral transcripts, TPA also induced morphological alterations of the cells. We observed a saturation density 25% higher than the control (uninfected TPA-treated) or infected (untreated) cultures 6 days after temporary TPA treatment (18 h). Furthermore, severa passages after TPA treatment, the BPV-1-infected cultures had acquired the ability to form colonies in medium having low serum content. This transformed phenotype was not evident in control cells.

We obtained similar results with a newly developed compound, 12-O-retinoylphorbol-13-acetate (RPA)²⁰, which combines the anti-promoting vitamin A with a phorbol este tumour promoter. RPA exerts a mitogenic effect on the skin o NMRI mice similar to that induced by TPA. When applied to BPV-1-infected MEF tissue culture cells, RPA activated the latent non-expressed viral genomes in a manner similar to TPA (Table 1).

However, not all cell lines provide a suitable environment for the above interactions (Table 1). While primate cells did no respond at all, a temporary activation of BPV-1 DNA replication in the absence of transcription was achieved by TPA

Table 1 Effect of TPA and RPA on BPV-1 episomes						
Cell type	Designation	DNA replication	Transcription	Transformation		
Monkey kidney	CV-1	-		_		
Human foreskin fibroblasts	HFF	_	_	_		
Human embryonic lung	HEL		~	_		
Bovine kidney	NBL	Transient	_	_		
Bovine fetal thyroid	BFT (ref. 21)	+	Transient	Transient		
Rat embryo fibroblast	REF	Transient	_	-		
Mouse embryo fibroblast	MEF*	+	+	+		

DNA replication and transcription were determined by the method of Southern¹⁶ 4 days after addition of 10⁻⁷ M TPA or RPA. Both compounds were applied separately to all cell types. Transformation reduced serum requirement and increased saturation density. –, No effect observed. 'Transient' denotes that the effect was abolished after two passages following TPA or RPA treatment. +, BPV-1 DNA replicates in the absence of tumour promoters.

^{*}DBA mouse strain, fifth passage; cells show a limited in vitro lifetime unless infected and treated with either TPA or RPA.

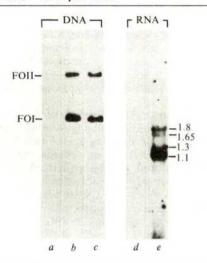


Fig. 2 Effect of RPA on BPV-1-infected BFT cells. BFT cells were infected with BPV-1 and passaged as described in Fig. 1 legend. Preparation and characterization of BPV-1-specific DNA and RNA sequences are also described in Fig. 1 legend. a-c, DNA from BFT cells. a, Uninfected; b, four passages after infection; c, as in b, but treated for 3 days with RPA (10^{-7} M) ; d, RNA from the same culture as in b; e, RNA from the same culture as in c. The values indicate the sizes of the BPV-1 RNA sequences in kb.

treatment of NBL cells. BFT cells, also of bovine origin, which are free of endogenous BPV-1 genomes (Fig. 2a), when infected with BPV-1 were capable of supporting the continuous replication of episomal BPV-1 DNA (Fig. 2b). However, no viral transcripts were observed (Fig. 2d) and a normal phenotype was retained. After exposure to RPA (or TPA, data not shown), no additional effect on the BPV-1 DNA content was observed (Fig. 2c), but transcription of the BPV-1 genomes was initiated (Fig. 2e) and the cells acquired a transformed phenotype (Fig. 3). However, these effects were transient, as transcription of the BPV-1 episomes was abolished after two further passages in the absence of promoter, despite the continuing replication of BPV-1 DNA. It is interesting that the relative amounts of BPV-1 transcripts obtained after RPA (and TPA) treatment corresponded initially to, or even exceeded those observed in BPV-1-transformed hamster embryo fibroblast (HEF) cells (Fig. 1j) which contain comparable numbers of episomal DNA, that is, ~50-100 per cell. Treatment with the tumour promoters failed to enhance the relative amount of BPV-1-specific transcripts in the latter case (not shown).

Thus, it seems that the promoter-treated BFT and MEF cells may constitute an appropriate host system for amplification and expression of foreign genes which have been inserted into a BPV-1 vector. Furthermore, the experimental system described here may be used for rapid screening of putative tumourpromoting agents, thereby reducing the need for time-consuming studies in laboratory animals.

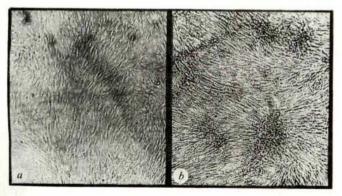


Fig. 3 Effect of RPA on BFT cells. BPV-1-infected cells (0.1 ml BPV-1 per 2×106 cells) were passaged (1:5 split ratio) 4 days after infection: a, 7 days after passaging in medium without RPA; b, parallel culture, but maintained for 7 days in medium containing 10⁻⁷ M RPA.

This work was supported by the Bundesminister für Forschung und Technologie. We thank Drs G. Fürstenberger, D. Berry, B. Sorg and F. Marks for the RPA, Dr E. Hecker for the TPA, and G. P. Gray for help in preparation of the manuscript.

Received 17 December 1981; accepted 4 March 1982.

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Electron microscopy of hepatitis B core antigen synthesized in E. coli

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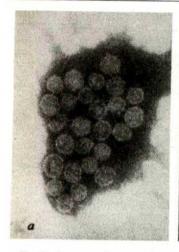
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DNA fragments from hepatitis B virus (HBV) have been inserted into plasmid vectors and cloned in Escherichia coli¹, and some of these bacterial clones have been reported to synthesize hepatitis B core antigen (HBcAg)1,2. Material from a derivative of one HBcAg-synthesizing clone is being evaluated in our laboratory and elsewhere^{3,4} as a serological reagent for use in various assays, including immune electron microscopy, to detect anti-HBcAg antibody. It has been shown by gel exclusion chromatography that the bacterial HBcAg is in an aggregated form3. We now report that the E. coli HBcAg preparation contains small round particles similar in appearance to the viral cores previously seen in material obtained from HBV-infected humans⁵⁻⁷ and chimpanzees⁸.

Extracts of E. coli harbouring the recombinant plasmid pRI-11 (ref. 3) and of human liver containing HBcAg were examined by immune electron microscopy (Fig. 1). The morphology of particles precipitated by anti-HBcAg antibody from the bacterial HBcAg preparation was similar to that of viral cores seen in extracts from HBV-infected liver. The average diameter of the particles from E. coli was 27.3 nm (range 24-31 nm, n = 100); that of particles in the liver extract was 28.1 nm (range 24–32 nm, n = 100).

Preliminary density gradient studies have shown that the E. coli- and liver-derived HBcAgs give bands at 1.35-1.36 g ml in caesium chloride, indicating that they contain nucleic acid; this was confirmed by the UV absorption spectra of both antigens (P. Wingfield and K. Murray, personal communication).

Further immune electron microscopy tests have confirmed that the particles seen in the E. coli preparation possess the characteristic antigenic properties of HBcAg. Results of these studies and of other confirmatory tests will be reported elsewhere.



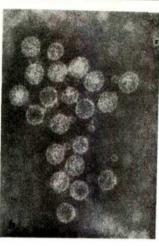


Fig. 1 Immune electron microscopy of a, E. coli-derived and b, human liver-derived HBcAg (×189,000). Each antigen (0.1 ml) was mixed with an equal volume of human anti-HBcAg IgG (2-400 µg ml⁻¹), incubated at 20 °C for 1 h, then diluted to 2.5 ml with phosphate-buffered saline and centrifuged at 48,000g for 1 h. The resulting pellet was resuspended in the minimum amount of distilled water (<0.1 ml) and mixed with an equal volume of negative stain (3% phosphotungstic acid, pH 6.3). A drop of the mixture was placed on a Formvar-carbon-coated grid which was then blotted dry with filter paper. The specimens were examined in an AEI 801 or a Jeol 100 CX electron microscope with magnifications calibrated using crystalline catalase

Electron microscopy has thus revealed the presence of small round particles in extracts of E. coli that carry a hybrid plasmid directing the synthesis of HBcAg. The morphological and antigenic properties of these bacterial HBcAg particles are comparable with those of naturally occurring HBV cores.

We thank Professor K. Murray and Biogen N.V. for providing E. coli extracts containing HBcAg, and Drs M. S. Pereira, A. M. Field and P. P. Mortimer and Professor K. Murray for helpful advice.

Received 27 January; accepted 4 March 1982.

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Tandem repeats in the N-terminal sequence of a proline-rich protein from corn endosperm

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Zeins, the prolamines of corn (Zea mays), are the most abundant storage protein fraction in corn endosperm. Like prolamines of other cereals, zeins are readily soluble in 60-70% alcohol solutions but are insoluble in aqueous buffer systems. The other major storage protein fraction in corn endosperm is glutelin, which is extractable with either dilute alkali or detergent. We have now extracted from corn endosperm a proline-

rich, zein-like protein fraction which we have purified by chromatography on phosphocellulose. Study of the N-terminal sequences of three chromatographic fractions for homologies shows that they are essentially identical. These N-terminal sequences include a hexapeptide Pro-Pro-Pro-Val-His-Leu, which is repeated in tandem at least six times, and probably eight times or more.

Moureaux and Landry1 and Paulis et al.2 independently discovered that some glutelin proteins were soluble in alcohol containing a disulphide reducing agent. This alcohol-soluble fraction of glutelin is referred to, amongst other names3-5 as alcohol-soluble reduced glutelin (ASG)6. Paulis and Wall7 showed that ASG could be separated into water-soluble and -insoluble fractions by dialysis against water. Water-insoluble ASG is similar to zein electrophoretically and in amino acid composition, whereas water-soluble ASG differs significantly. Water-soluble ASG constitutes ~5% of total protein in the corn endosperm (unpublished data).

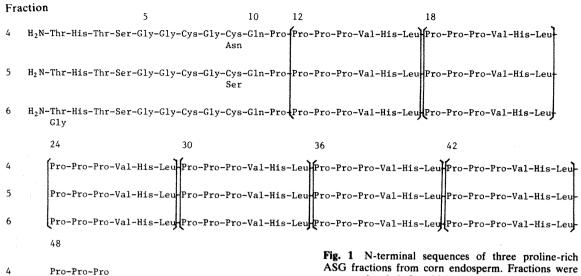
We recently⁸ fractionated ASG by chromatography on phosphocellulose and found that three late-eluting fractions, fractions 4 (peak 4), 5 (peak 5) and 6 (the late-eluting tail of peak 5), contained proteins similar to either the water-soluble ASG or the reduced-soluble (RS) protein described by Wilson et al.9. The amino acid compositions of these ASG fractions are essentially identical, but differ significantly from zein8. They contain a large number of proline residues (~26 per 100 residues) and are exceeded in proline content only by certain proteins in wheat omega-gliadin¹⁰, barley C-hordein¹¹ and human saliva¹². SDSpolyacrylamide gel electrophoresis showed that all three fractions are homogeneous and have a molecular weight (M_r) of 27,500. In contrast, they show extensive charge heterogeneity (14-16 components, with 5 predominant ones) on isoelectric focusing (IEF). All three IEF profiles are essentially similar, however, the differences being primarily quantitative.

N-terminal amono acid sequence analysis revealed that these proline-rich ASG fractions have identical sequences for at least 49 residues (Fig. 1). This degree of sequence homology suggests that the polypeptides are products of homologous genes which arose by duplication from an ancestral gene. Major heterogeneity was evident at only two positions (1 and 9). Glycine and threonine were found in an approximately equimolar ratio at residue 1 of fraction 6; at residue 9, in addition to cysteine, asparagine was detected in fraction 4 (Cys/Asn~1:1) and serine in fraction 5 (Cys/Ser ~1:2). The presence of serine and cysteine at the same position can be accounted for by a point mutation involving a single nucleotide substitution. In addition to positions 1 and 9, slight microheterogeneity was occasionally noted in the first nine positions, amounting to no more than 5-10% of the major determined residue. This may explain the heterogeneity of these fractions observed after IEF. There was no sequence heterogeneity apparent from residues 10-49, indicating possible evolutionary pressure for conservation of this sequence.

The N-terminal sequence of proline-rich ASG fractions 4-6 is extremely hydrophobic: 69% of the amino acids identified in the first 58 positions are proline, valine and leucine. In fact, proline accounts for 43% of the first 58 residues. On the basis of amino acid composition and estimated molecular weight8, it is expected that fractions 4-6 contain ~60 prolines out of a total of 225-230 residues, and the occurrence of 25 prolines in the first 58 positions indicates an uneven distribution of this amino acid. This is also true for histidine, where 9 (of 12-14 total expected) histidine residues occur in the first quarter of the molecule, making its N-terminus positively charged. Indeed, the occurrence of regularly spaced histidines in a region rich in proline. valine and leucine may be responsible for the solubility of fractions 4-6 in water, as charge repulsion between histidines could diminish hydrophobic interactions among nonpolar residues.

The most surprising observation from the data in Fig. 1 is that a hexapeptide, Pro-Pro-Pro-Val-His-Leu, is tandemly repeated at least six times, and probably a minimum of eight times, in

6



fractions 4-6, starting at position 12. This is the first report of so many identical tandem repeats of such long unit length occurring in a plant protein. There is some evidence that Tripsacum aestivum and Tripsacum monococcum gliadins and barley C-hordein, which are proline-rich, contain repeating pentapeptide sequences (D. D. Kasarda, personal communication).

Pro-Pro-

54

Pro-Pro-Pro-Val-His- X -Pro-Pro-Pro-Val-His-

Periodicity in amino acid sequences of some animal proteins (for example, collagen, keratin, silk fibroin, protamine and tropoelastin) has long been known 13-15. Identical tandem repeats, however, have been found in only a few proteins, most notably in freezng point-depressing glycoproteins 16 and tropoelastin 15,17. In tropoelastin, 4-, 5- and 6-residue-long units were found to be repeated in tandem in different peptides. Nontandem identical repeats have also been reported in proteins isolated from human saliva12,18

To determine whether the repeating hexapeptide Pro-Pro-Pro-Val-His-Leu from fractions 4-6 shows any sequence similarity to other proteins of plant or animal origin, the National Biomedical Research Foundation undertook a computer search for this repeating unit in all proteins for which sequence data were available, from mid-1980. Of 164,931 6-residue-long sequences from 1,439 proteins, none was identical to the repeating unit found in ASG fractions 4-6.

The presence of essentially a single N-terminal sequence in proline-rich ASG fractions was rather surprising in view of the extensive charge heterogeneity detectable by IEF in these fractions. Furthermore, it is widely believed that seed storage proteins are relatively free from selective pressures and thus can readily tolerate and accumulate random amino acid substitutions¹⁹⁻²¹. It is also surprising that 6-residue-long tandem repeats have identical sequences, with little indication of divergence of any repeating units. The invariance of this repeating sequence may be due to the following. Proline-rich proteins may have an important, presently undefined, biological function that places constraints on variations in their primary structures. This may not be tenable, however, in view of the fact that some inbred maize strains (for example, W64A) lack proline-rich ASG proteins and have no detectable abnormalities. Alternatively, duplication and divergence of the homologous prolinerich ASG genes from their ancestral gene may have occurred recently. This is the more plausible explanation as archaeological and palaeontological data suggest that corn was domesticated only 5,000-10,000 years ago, and that a wild corn existed

converted to their S-pyridylethyl derivatives and subjected to automated Edman degradations on a Beckman 890C sequencer, using 1M Quadrol programs 122,974 and 111,978 (ref. 21). The resulting phenylthiohydantoin amino acids were identified by HPLC and two-dimensional TLC.

as long as 80,000 years ago²². DNA hybridization data, however, suggest that early corn or its immediate wild ancestor may have diverged from its nearest possible progenitor, teosinte, 10-20 million years ago, and from its other possible progenitor, Tripsacum, 40 million years ago²³.

Obviously, tandem 6-residue repeats in proline-rich ASG fractions are colinear to 18-nucleotide-long DNA repeats. These DNA repeats may have arisen either by saltatory replication²⁴, by unequal crossing-over²⁵, or by one of the other mechanisms reviewed elsewhere²⁶. In fact, we postulate that repeats in proline-rich ASG represent an insertion into an ancestral prolamine gene from one of the repetitive sequence families of the maize genome, rather than from intracistronic tandem duplications involving an 18-nucleotide sequence. The isolation of mRNAS encoding proline-rich ASG polypeptides, the production of cDNA probes from these mRNAs, and hybridization of the probes with various components of maize DNA may elucidate the origin of these repeated sequences.

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Activation of transcription of a yeast gene in *E. coli* by an IS5 element

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pRG4 and pRG5 are two plasmids derived from pBR322 which carry an integrated yeast DNA segment (2.5 kilobases) containing the *URA1* gene^{2,3} and differ only in the orientation of the insert. Escherichia coli pyrD cells transformed by pRG4 recover the ability to grow on minimal medium whereas pRG5transformed pyrD cells do not3. The spontaneous integration of an insertion (IS) element into the cloned DNA segment corrects the absence of complementation in pRG5-transformed pyrD cells3. The new plasmid resulting from such an insertion event has been named pRG7 (Fig. 1). To determine the level at which the IS element affected URA1 expression, we assayed the RNAs which hybridized to each strand of URA1 DNA in various transformed pyrD strains, using as a probe single-stranded URA1 DNA cloned in phage fd106⁴. The results showed that the switching on of yeast gene by the IS element occurred at the transcriptional level, strongly suggesting that this IS element can serve as a mobile promoter in E. coli. The IS element has been identified as an IS5.

To determine which strand served as a template for the transcription of *URA1*, we cloned the 2.5-kilobase (kb) fragment containing *URA1* into the *Hin*dIII site of the replicative form of phage fd106^{4.5}. Phages carrying the yeast DNA in both directions were obtained from infected KB35F⁺ cells⁴. DNA from each type of phage was used to assay the *URA1* mRNAs in the two isogeneic yeast strains FL100 and *ppr1*-1⁶, a strain

Table 1 Transcription of the URA1 region in yeast

Yeast strain		Amount of RNA hybridized to the plus strand of URA1 DNA	Amount of RNA hybridized to the minus strand of URA1 DNA	
Expt 1 Expt 2	Wild-type FL100 ppr1-1 Wild-type FL100 ppr1-1	$ \begin{array}{c} 1.2 \times 10^{-5} \\ 6.8 \times 10^{-5} \\ 3.3 \times 10^{-5} \\ 15.3 \times 10^{-5} \end{array} $	$0.3 \times 10^{-5} \\ 0.4 \times 10^{-5} \\ 0.5 \times 10^{-5} \\ 0.9 \times 10^{-5}$	

Yeast cells were grown in minimal medium (Difco yeast nitrogen base 6.7 g Γ^{-1} , 2% glucose) at 30 °C. Cells were labelled in exponential phase using ³H-adenine (20 μ Ci m Γ^{-1}) of specific activity 17 Ci mmol $^{-1}$. Yeast RNAs were extracted from 10-ml cultures ^{15.16}. Conditions for hybridization of RNA to DNA fixed on nitrocellulose filters (Sartorius) are described in ref. 16. The nitrocellulose filters carrying probe DNA were prepared according to ref. 17. Specific hybridization of the cloned yeast DNA was measured in triplicate as follows: labelled RNA was incubated in a small glass vial with one filter loaded with 1 μ g fd-ural plus strand DNA and one filter loaded with 1 μ g of fd106 DNA. The strand which hybridized the most RNA was arbitrarily named the plus strand. To evaluate hybridization to each strand of the yeast DNA, c.p.m. retained on a fd DNA filter were subtracted from c.p.m. on a fd-ural DNA filter. The amount of hybridization was expressed as the fraction of radioactivity specifically retained versus the input. Conditions were such that the input of radioactivity per unit cell mass was the same for both strains. Expts 1 and 2 are two completely independent experiments.

constitutive for synthesis of dihydroorotate dehydrogenase. The regulatory mutation ppr1-1 affects transcription of the unlinked gene URA1, the level of URA1 mRNA being about five times higher in the ppr1-1 strain than that in the wild-type strain². The results presented in Table 1 indicated that in the wild-type strain yeast RNA hybridized to both strands of the cloned yeast DNA. About 83% of these hybridized RNAs were complementary to one strand, arbitrarily named plus, 17% to the other strand named minus. In the ppr1-1 strain, the level of the RNA hybridizing to the plus strand was about five times higher than that found in the wild-type strain, whereas the amount of RNA hybridizing to the other remained almost unchanged, showing that the plus strand carried the sequence complementary to URA1 mRNA. Hybridization to both strands of the 2.5-kb cloned DNA was still observed after subsequent purifications of the plus phage and thus could not be attributed to contamination of the plus strand by the minus strand. Rather, this suggested that the cloned fragment not only encompassed the entire URA1 sequence but additionally contained the end of a second

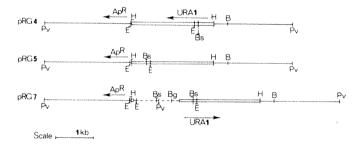


Fig. 1 Structure of plasmids pRG4, pRG5 and pRG7. The construction of these plasmids is described in refs 2 and 3. The DNAs are represented linearized after single cleavage at the PvuII site of pBR322¹. pBR322 DNA sequences are represented by a continuous single line, yeast DNA sequences by an open box and the IS element DNA by a broken single line. The sites shown for restriction endonucleases cleavage are as follows: E, EcoRI; H, HindII; B, BamHI; Bs, BstEII; Bg, BgIII; Pv, PvuII. The arrows indicate the direction of transcription of the ampicillin-resistance (Ap^R) gene¹ and of the URA1 gene. kb, Kilobase.

gene in the opposite strand. The orientation of the *URA1* region in each type of phage was determined by *EcoRI* restriction analysis of the corresponding replicative forms (not shown). The polarity of the virus strand being known⁴ and the template strand for *URA1* transcription determined to be the plus strand, the direction of transcription of *URA1* could be deduced to proceed from the internal *EcoRI* site towards the distal *HindIII* site (see Fig. 1).

We then assayed the RNAs which hybridized to each separated strand of the cloned yeast DNA in the E. coli pyrD strains transformed by pRG4, pRG5 or pRG7 (Table 2). In the pRG5-transformed pyrD strain, no dihydroorotate dehydrogenase activity was found, in agreement with our previous results3; no hybridization of RNA to the plus strand could be detected in typical pulse-label experiments, indicating that transcription of URA1 did not occur in this strain. One could propose two explanations for this absence of transcription. The first would be that there is a signal somewhere in the 5' end of the cloned gene that is recognized as a stop by the E. coli RNA polymerase and therefore impedes the transcription of URA1, an hypothesis which can be discarded because URA1 is transcribed when cloned in the opposite direction. The second explanation would be that no yeast sequence in this cloned segment is recognized as a promoter by the E. coli RNA polymerase; the transcription of URA1 would therefore depend on the existence of a pBR322 promoter and would only

occur by a readthrough mechanism. In support of this hypothesis, Stüber and Bujard have found that the ampicillinresistance gene of pBR322 could be transcribed from two promoters, one of which is located near the HindIII site and can serve to initiate the transcription of DNA segments inserted into this site. Notably, the direction of transcription of URA1 in pRG4 coincides with that of the ampicillin-resistance operon. No pBR322 promoter can be used to transcribe URA1 in pRG5, as the promoter of the tetracycline-resistance operon is inactivated by HindIII cleavage7. However, when URA1 is integrated into the BamHI site, which is located inside the structural gene for tetracycline resistance, again only one orientation of the cloned fragment allows expression of the URA1 gene3. In this case, the direction of transcription of URA1 coincides with that of the tetracycline-resistance operon. These results strongly support the hypothesis that URA1 requires a bacterial promoter for its expression in E. coli. However, it is surprising that the minus strand in the pRG5-transformed strain is very poorly expressed compared with the plus strand in the pRG4-transformed strain, although involvement of the same pBR322 promoter in both cases is suspected. This might imply that the minus strand contains sequences which signal inhibition of transcription in E. coli.

The interesting point is that in the pRG7-transformed strain, the transcription of URA1 is restored. This indicates that the integration of the IS element provides the promoter activity required by URA1 for its transcription in E. coli. A restriction map of this IS element shows that EcoRI, BglII, PvuII and BstEII cleave within the insertion, whereas BamHI and HindIII do not. According to this map, the size of the IS element is

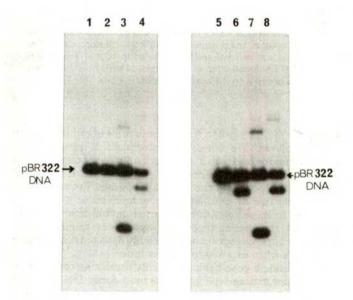


Fig. 2 Hybridization of the IS element controlling the expression of URA1 to IS5. pDI14 is a pBR322 plasmid carrying a 3-kilobase insert of Haemophilus haemolyticus DNA at the PstI site. pDI40 only differs from pDI14 in having an IS5 element integrated inside the *H. haemolyticus* DNA segment. The structures of these plasmids are described in ref. 9. The DNAs run on 0.8% agarose gels were as follows: lanes 1 and 5, ~4 ng of HindIII-digested pRG5 DNA; lanes 2 and 6, ~4 ng of HindIII-digested pRG7 DNA; lanes 3 and 7, ~4 ng of PstI-digested pDI14 DNA; lanes 4 and 8, ~4 ng of PstI-digested pDI40 DNA. After electrophoresis, the DNAs were transferred to a nitrocellulose filter¹⁰. Native pDI14 and pDI40 DNAs were made radioactive by 'nick-translation' (specific activity of each DNA was ~107 c.p.m. µg lated pDI14 DNA was hybridized to the transferred DNAs of lanes 1-4 and nick-translated pDI40 DNA was hybridized to the transferred DNAs of lanes 5-8. The HindIII insert of pRG5 DNA hybridizes neither to pDI14 DNA nor to pDI40 DNAs; the HindIII insert of pRG7 DNA does not hybridize to pDI14 DNA but does hybridize to pDI40 DNA.

Table 2 Expression of the URA1 region in E. coli pyrD strains transformed by various plasmids

Strain	Plasmid	Dihydroorotate dehydrogenase activity	Amount of RNA hybridized to the plus strand of URA1 DNA*		
E. coli pyrD	pRG4 pRG5 pRG7	7.2 <0.01 7.5	200×10^{-5} $< 10^{-5}$ 217×10^{-5}	2.7×10^{-5} 2.2×10^{-5} 2.3×10^{-5}	

E. coli pyrD cells were grown at 37 °C in minimal medium containing ampicillin (50 μ g ml⁻¹) and uracil (20 μ g ml⁻¹). The dihydroorotate dehydrogenase activity was assayed as previously described using an acellular extract from a 100-ml culture of exponentially growing cells. The activity is expressed in nmol of substrate transformed per min per mg protein.

A 10-ml culture of exponentially growing cells was labelled for 2 min with ³H-adenine (10 μCi ml⁻¹) of specific activity 17 Ci mmol⁻¹ Crude E. coli extracts were obtained by sonication. Conditions for hybridization were the same as in Table 1. The amount of hybridization was expressed as the fraction of radioactivity specifically retained versus the input.

actually ~1.2 kb and not 0.9 kb as previously estimated3. Our IS element has been shown to hybridize to IS5 (1,195 base pairs)8. using the Southern procedure 10 (Fig. 2).

Until now, only IS2 elements, and in one case reported recently11 an IS3 element, have been observed to activate bacterial gene expression in E. coli (see ref. 12 for a review). IS1 and IS5 elements were also found to activate the cryptic bgl operon in E. coli K-12, but in this case the IS elements served to disrupt an operator site rather than provide a promoter sequence13. IS2 was also shown to increase the expression of a cloned yeast gene (TRP5) in E. coli14. This is the first time that an IS5 element has been reported to exert a transcriptional effect on the expression of a cloned yeast gene.

It is not clear whether a sequence carried by this IS5 element is in itself sufficient to bestow promoter activity. For instance, some region(s) of the IS5 element might function as part of a promoter which would also involve yeast or/and even additional upstream pBR322 sequences. Such a possibility has already been discussed by Glandsdorff et al.11 in the case of IS2dependent gene activation.

This work was carried out in Professor F. Lacroute's laboratory, and we thank him for his interest in our work. We also thank Drs P. Starlinger and B. Schoner for sending us various cloned IS element DNAs, and Dr J. C. Hubert and Mrs Nguyen-Juilleret for help in some experiments. G.L. was supported by a Rhône-Poulenc fellowship.

Received 14 December 1981; accepted 2 March 1982.

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Molecular rearrangement of mating-type genes in fission yeast

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In the wild-type homothallic strain (h^{90}) of the fission yeast Schizosaccharomyces pombe, the mating type of a cell changes at almost every cell division1. Genetic analysis has suggested that mating-type switching results from copy transposition of genes from one of two storage cassettes into an active site2. This model is functionally similar to that already established for Saccharomyces cerevisiae3 but is different in that the three matingtype genes involved are tightly linked in S. pombe. Heterothallic strains in which mating-type switching is much reduced or absent arise from the homothallic wild type either through the irreversible loss of one of the silent storage cassettes (generating heterothallic stable minus strain h^{-s}) or through a genetic change which has been formally interpreted as a change in the switching signal at the mating-type locus (generating heterothallic normal plus (h^{+N}) and unstable minus (h^{-U}) strains. We have now isolated one of the mating-type genes mat-P, and have used it as a hybridization probe for sequence changes at the mating-type locus. We confirm that the genetic loss suggested for h^{-s} is due to deletion and demonstrate that the impairment of switching in h^{+N} and h^{-U} is correlated with a duplication.

The genetics of the mating-type locus are summarized in Fig. 1. To test this model at the molecular level, we have used the newly developed techniques for genetic transformation of S. pombe⁴ to isolate a functional copy of the mat-P gene from a

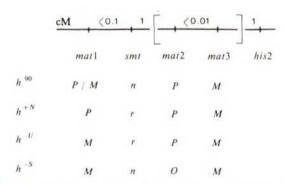


Fig. 1 Genetic map of chromosome II in the vicinity of the mating-type locus and the allocation of mating-type alleles to homothallic (h^{90}) and heterothallic h^{+N} , h^{-U} and h^{-S} strains of $S.\ pombe^{5}$. The momentary allele present at mat1, P (plus) or M (minus), determines the cellular mating type and has been derived by transposition from one of the two silent storage genes, mat2-P or mat3-M during the previous event of mating-type switching. The normal switching signal smt-n (a cis-acting sequence) of the homothallic strain h^{90} can change to smt-r, which reduces switching frequencies from $>10^{-1}$ to 10^{-4} or lower, giving rise to the slowly interconverting heterothallic strains h^{+N} and h^{-U} . The other heterothallic strain h^{-S} is presumed to be completely stable because it has lost the silent P information stored in mat2 (its switching signal is still normal and can be recovered by recombination). The mat3 gene was referred to as 'matX' in ref. 2. It has since been mapped to the vicinity of mat2 (R.E., unpublished results), but the relative order of mat2 and mat3 has not yet been determined.

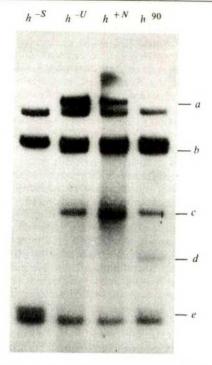


Fig. 2 Autoradiograph of 32 P-labelled pMT3.2 (nick-translated) hybridized to a Southern transfer of EcoRI-digested DNA of four mating-type strains, h^{-S} , h^{-U} , h^{+N} and h^{90} . All four strains are essentially isogeneic. $972 \ h^{-S}$ and $975 \ h^{+N}$ were obtained originally from Urs Leupold, and h^{-U} and h^{90} were isolated as spontaneous derivatives from h^{+N} before this experiment. DNA was prepared by lysis of protoplasts in 50 mM Tris pH7.6, 50 mM EDTA, 2% SDS at 65°C. The lysate was extracted with phenol, then chloroform, and precipitated with 0.5 vol of isopropanol. The precipitate was pelleted and resuspended in 10 mM Tris pH7.6, 1 mM EDTA. Small samples were digested with EcoRI and electrophoresed on 0.8% agarose gel at 2 V cm $^{-1}$ for 18 h.

gene bank of $S.\ pombe$ DNA. The strategy for isolating this gene was as follows. We constructed a diploid $S.\ pombe$ strain homozygous for $leu\,1^-\ his\,2^-\ h^{-S}$; such a strain contains only the mat-M gene and is therefore unable to sporulate. If the mat-P gene is introduced into the cell via a plasmid, the cell should become capable of sporulation once deprived of a source of nitrogen. In these conditions a normal diploid that is heterozygous at the mating-type locus sporulates spontaneously. Colonies containing spores can be readily detected because they stain black when exposed to iodine vapour.

To generate a gene bank potentially containing copies of all the different mating-type genes, we used donor DNA derived from the three mating-type strains h^{90} , h^{+N} and h^{-S} . These DNAs were mixed, partially digested with Sau3A and ligated to the unique BamH1 site in the tetracycline-resistance gene of the hybrid yeast/bacterial cloning vector pDB248 (ref. 4). pDB248 consists of the bacterial vector pBR322, the S. cerevisiae leu2+ gene which allows selection in leu1.32 strains of S. pombe and part of the 2 µm plasmid carrying the replication origin. A bank of recombinant molecules was generated in Escherichia coli and used to transform the diploid S. pombe strain, homozygous for leu1 his2 h-s. Transformants were selected as leu+ prototrophs on sorbitol-containing plates lacking leucine, at a frequency of ~1 in 2,000 viable protoplasts and 20,000 per µg of plasmid DNA. On exposure of the colonies to iodine vapour, 34 of the 70,000-80,000 transformants reacted with iodine and were found to contain spores and asci. The plasmids producing sporulating transformants were candidates for carrying an insert of mat-P sequences.

Three transformants were further analysed and found to be unstable. leu1+ and mat-P were lost from ~20% of the cells

even when clones were grown under leucine selection and both genes were almost always lost together, indicating that mat-P is linked to leu1+ on the unstable autonomously replicating cloning vector. Spores derived from the mat-P transformants were germinated and 48% of the resultant colonies were found to be leu1+ and iodine positive, although the extent of staining was variable. Some clones were found to be stable for both leu 1 and mat-P presumably as a consequence of integration into the chromosome. To determine whether this occurs at the matingtype locus, several haploids were crossed to a leu1 h+Nhis2 strain for tetrad analysis. Only one cross-over between leu 1 and his 2 was observed in a total of 81 tetrads with 4 germinating spores. As leu1 is normally 20 centimorgans (cM) from his2, their close linkage of <1 cM indicates that the plasmids have integrated at the mating-type locus adjacent to his 2. Furthermore, the h^{-S} haploid containing the integrated copy of the plasmid was found to have regained the mating-type switching properties of the h^{90} strain. This suggests that integration may have occurred at mat2, the silent store of mat-P information, as integration at mat1 would presumably result in immediate loss of mat-P during subsequent switching.

One of the mat-P plasmids was recovered from a yeast clone in which integration had not occurred, by re-transformation of E. coli (D.B., M. Piper & P.N., unpublished results). This plasmid, pMT3.2, was able to confer sporulating ability on diploid and haploid h^{-S} and on haploid h^{-U} strains, but not on h^{+N} . It therefore carries a functional copy of mat-P but not mat-M. The plasmid pMT3.2 was digested with EcoRI and the lengths of the fragments generated were measured. The insert into the BamHI site of the vector was found to be ~ 10 kilobases (kb) and to contain one EcoRI site. As the cloning vector pDB248 has no detectable sequence homology with the S. pombe genome, pMT3.2 can be used directly as a hybridization probe to analyse the chromosomal organization of the mating-type locus. DNA from the four mating-type strains h^{-s} , h^{-U} , h^{+N} and h^{90} was digested with EcoRI and hybridized with nick-translated pMT3.2 using the Southern procedure (Fig. 2). The most striking features of the hybridization pattern are as follows. Although the S. pombe insert in pMT3.2 contains only one EcoRI site, the probe hybridizes to six bands in the h strain, and thus some of the insert sequences must be present in more than one copy within the genome. Compared with h^{90} , the lacks three bands b, c and d (Fig. 2) at 5.4, 4.0 and 3.0 kb, respectively, while one small new band (Fig. 2, 1.7 kb) is apparent. On the other hand, in both h^{+N} and h^{-U} there is a prominent extra band (a, 7.2 kb) and loss of one weakly hybridizing band (d, 3.0 kb) compared with h^s

The simplest interpretation of these data is that h^{-s} arose from h^{90} by loss of the silent mat-P cassette, thereby generating a strain which never reverts to h^{90} and lacks certain restriction fragments. The bands which h^{-S} and h^{90} have in common may reflect sequence homology between the mat-P and mat-M genes, but some hybridization may be due to sequences adjacent to the mat-P gene which are not directly involved in mating-type determination. h^{+N} and h^{-U} carry a duplication of mating-type information. This may have occurred in two different ways: it could result from either a faulty transposition event in which the resident gene at mat1 was not excised during insertion of a new gene from the silent store, or unequal crossing-over between sister chromatids at the mating-type locus. If a copy of mat-P at the mat1 site recombined with the same gene at mat2 on the sister chromatid, the products would be a cell which had a duplication of mat-P and the other a deletion of mat-P. This model predicts that the arisal of h^{+N} and h^{-S} from h^{90} is a reciprocal event.

The duplication in h^{+N} and h^{-U} might be expected to reduce the rate of switching. When the duplication is removed there will be a reversion to h^{90} ; this occurs at a frequency of 5×10^{-4} . The Southern blot hybridization patterns do not distinguish between h^{+N} and h^{-U} although they presumably contain different mat 1 genes. This suggests that the region which differs between mat1-P and mat1-M is not cut by EcoRI and is of sufficiently

similar length as to generate bands that are indistinguishable on a gel.

Our results thus indicate the way in which heterothallic strains arise in S. pombe. They bear only indirectly on the mechanism of homothallic mating-type switching in this yeast but the isolation of the first hybridization probe for the mating-type locus opens the way for a more refined analysis of this process.

We thank Melanie Piper, Rachel McIntosh and Tove Friis for technical assistance, Sydney Shall for laboratory facilities, and Urs Leupold for helpful discussions. This work was supported by the CRC (D.B.), SERC (P.N.) and EMBO (R.E.).

Received 18 September 1981; accepted 24 February 1982.

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The iron-oxygen bond in human oxyhaemoglobin

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Knowledge of the exact nature of the iron-oxygen bond in oxyhaemoglobin (oxyHb) is essential for the understanding of cooperative oxygen binding to haemoglobin (Hb)1. However, X-ray studies of oxyHb have previously been hindered by the tendency of oxyHb crystals to autoxidize. Here we report the stereochemistry of the haem-oxygen complex in human oxyHb, as determined by single-crystal X-ray analysis. Bent end-on geometry of the iron-oxygen bond in haem proteins, as predicted by Pauling², was first observed in the 'picket fence' complex³ and has since been observed in oxymyoglobin (oxyMb)4,5; in oxyerythrocruorin, however, the Fe-O-O bond is almost linear⁶. In oxyHb the Fe-O-O bond angle is 156°, intermediate between the 'picket fence' compound and erythrocruorin. The position of N^e of His E7 in the α -subunit suggests that it forms a hydrogen bond with the bound oxygen, as in oxyMb⁷. In the β -subunit, however, N° of His E7 is located further from the oxygen, suggesting that the hydrogen bond is weaker.

Crystals of human oxyHb were grown according to Perutz⁸, with minor modifications to retard autoxidation. Reflections to 2.1 Å resolution were recorded with an Arndt-Wonacott oscillation camera at -2 °C. After exposure, the crystals were dissolved and spectra obtained to determine the level of methaemoglobin (MetHb). None of the crystals used contained more than 10% MetHb. Details of crystallization, data collection and processing will be published elsewhere.

The human carbomonoxyhaemoglobin (HbCO) model¹⁰ served as a starting point for refinement, using the Jack-Levitt combined X-ray and energy method¹¹. At present, the agreement between observed and calculated structure factors, expressed as a conventional R factor, is 0.22. Positional standard deviations of atoms were estimated using a least-squares matrix⁵ and Luzzati plot¹².

Table 1 summarizes the stereochemistry of the haem-oxygen complexes in oxyHb, oxyMb and the 'picket fence' compounds. Figure 1 shows the striking difference between the Fe—O—O bond angles in oxyHb and oxyMb, that is, 156° (± 10) in both subunits of oxyHb compared with 115° (±5) in oxyMb⁵. This results from differences (0.5-1.5 Å) in the location of the distal residues (that is, His E7, Val E11 and Phe CD1) in contact with the ligand. Because of a change in position of the E helix and CD

Table 1 Geometry of haem-oxygen complexes in oxyHb, oxyMb and picket fence compounds

	Fe—Ct(Å)	Fe-N(porph) (Å)*	Fe-N ^e (F8) (Å)	Fe-O1 (Å)	F8-N1FeN3(°)
OxyHb (\alpha-subunit)	0.13(8)	1.98(4)	1.95(10)	1.67(8)	11
OxyHb (β-subunit)	-0.08(8)	1.96(9)	2.06 (9)	1.83 (13)	27
OxyMb (ref. 5)	0.18(3)	1.95 (6)	2.07 (6)	1.83 (6)	1
FeO ₂ (TpivPP)(1 MeIm) (ref. 19)†	-0.03	1.98(1)	2.07(2)	1.75(2)	20
FeO ₂ (TpivPP) (2 MeIm) (ref. 19)†	0.086	1.996 (4)	2.107 (4)	1.898 (7)	22

Fe-Ct, distance of Fe from mean plane of porphyrin nitrogens; positive value denotes displacement towards His F8. F8-N1FeN3, angle between imidazole plane of His F8 and line N1-Fe-N3 (vertical line across haem in Fig. 1).

^{† &#}x27;Picket fence' compounds. TpivPP, meso-tetrakis[$\alpha,\alpha,\alpha,\alpha$ -[(O-pivaloyl)amido]phenyl] porphyrin; 1 MeIm, 1-methylimidazole; 2 MeIm, 2methylimidazole.

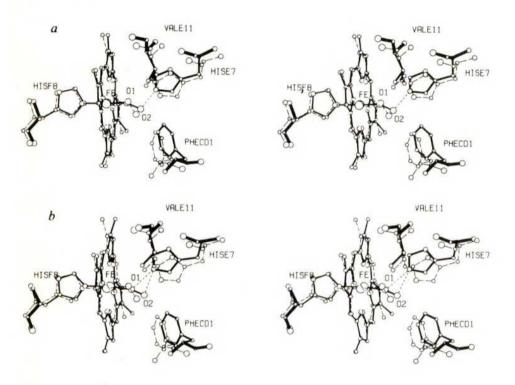


Fig. 1 Stereo view of superimposed haem environments oxyHb subunits and oxyMb. Haems were superimposed by least-squares fit and the other residues transformed accordingly. Thick lines denote oxyHb and broken lines, oxyMb. Note the upward movement of the terminal oxygen in oxyHb, following the distal residues. a, a-Subunit of oxyHb and oxyMb. Dotted line indicates a probable hydrogen bond, N^{ϵ} (His E7)—O2. b, β -Subunit of oxyHb and oxyMb: Nº is approximately equidistant from

O1 and O2 (dotted lines).

corner in oxyHb (see Fig. 1), these residues are further from the haem centre than in oxyMb. Thus, the haem pocket of oxyHb is more spacious than that of oxyMb. Extended Hückel calculations have shown that the iron-oxygen bond can vary between 120° and 160°, with only small consequent changes in energy (160° is, however, a favourable angle)^{13,14}. Hence, the more obtuse Fe-O-O bond angle observed in oxyHb may reflect either a reduction in steric constraints imposed upon the bound oxygen, or a different set of constraints.

Because of its loosely packed haem pocket, R-state Hb15 can accommodate CO, which binds to iron in a linear mode, more easily than Mb. In fact, the terminal oxygen in oxyHb (O2 in Fig. is close to the position occupied by the CO oxygen in HbCO. R-state Hb, therefore, has a higher O2/CO partition coefficient than Mb, which results in reduced O2/CO discrimination (the partition coefficient, $M = P_{1/2}O_2/P_{1/2}CO$, for *R*-state Hb is 200-250; that for Mb is 25-40)¹⁶.

Recently, Phillips and Schoenborn demonstrated by neutron diffraction that N" of His E7 forms a hydrogen bond with the terminal oxygen in oxyMb. In the α -subunit of oxyHb, N*—O2 is 2.7 Å and the geometry favours a similar hydrogen bond (Fig. 1a). In the β -subunit, N^{ϵ} of His E7 is approximately equidistant from O2 and O1 (3.4 Å and 3.2 Å, respectively; see Fig. 1b), the larger distance implying the presence of a weaker hydrogen bond with one or both oxygen atoms. Studies on the rate of formation of MetHb from oxyHb have shown that the α -subunit autoxidizes faster than the β -subunit^{17–18}. By forming a stronger hydrogen bond, the oxygen molecule in the α -subunit may become more polarized, thus allowing more rapid autoxidation.

The detailed structure of human oxyHb will be published elsewhere.

I thank Dr M. F. Perutz for suggesting the project and for continuing encouragement. I thank EMBO for a long-term fellowship.

Received 30 November 1981; accepted 25 February 1982.

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BOOK REVIEWS

Physicist extraordinary

Rudolf Peierls

MARK Oliphant is one of the great personalities of the world of physics, whose career has had many very different facets. Original research in nuclear physics in the Cavendish Laboratory under, and in close collaboration with, Rutherford, was followed by leadership of the radar team at Birmingham which produced the cavity magnetron and by work with Lawrence at Berkeley on the electromagnetic isotope separation for the atom bomb. After the War came the idea of the synchrotron, conceived independently and probably before others, and the creation of a nuclear physics school at Birmingham, with the construction of a cyclotron and a proton synchrotron; then, after the return to Australia, a major share in the development of the National University and, after retirement from the laboratory, a term as Governor of his native state of South

But no list of this kind could bring out Oliphant's most characteristic quality, the fearlessness with which he speaks his mind, no matter whether his thoughts are popular or unpopular, never calculating their effect on his image. While therefore what he says and writes does not always please his listeners, they always respect his intrinsic honesty. This quality came out perhaps most strongly, and created the greatest surprise, in his last, most exalted position as Governor. The men chosen for such representative positions are often senior statesmen or military men, very conscious of protocol, and maybe stuffy. Stuffiness is a quality completely lacking in Oliphant, and his direct and outspoken ways went over well in a country where stuffiness is not welcomed.

He speaks with equal directness about his failures and errors of judgement, and this has made it easier for the authors to write this biography in his lifetime. Writing about living people who can answer back, can be embarrassing, but evidently not in this case.

The authors have interviewed Oliphant and members of his family, as well as many colleagues in his different spheres, and they have had access to many letters and other documents. As a result the book brings out Oliphant's personality vividly. Perhaps the best part of the book is the account of his ten years in Cambridge. The authors do not give much detail of his papers on nuclear physics, but then they are not writing for physicists. What comes out so well is the atmosphere in the Cavendish, and Rutherford comes completely alive. Oliphant was perhaps Rutherford's

Oliphant: The Life and Times of Sir Mark Oliphant. By Stewart Cockburn and David Ellyard. Pp.369. ISBN 0-9594164-0-4. (Axiom Books, Australia: 1981.) A\$19.95.



Oliphant in 1950, the year of his move from Birmingham to Canberra.

favourite pupil, and we understand why. The characters of these two men had much in common in their directness, and in their enthusiasm for the work. A lecture by Rutherford had made a deep impression on Oliphant when still a student, and had led to his resolve to get to Cambridge and work under Rutherford; the great respect and awe gradually gave way to genuine affection.

Yet he left Cambridge, as other collaborators of Rutherford had done, to branch out on his own in Birmingham. He took up his new post just before Rutherford's death in 1937. His intention was to build up nuclear physics in Birmingham, but the process was soon interrupted by the War. The book describes the radar work, and his abortive trip to Australia to offer his services to the Australian government when the radar problems at Birmingham had lost their urgency and the war in the Pacific was approaching his native country. Finding that no one was prepared to arrange for his services to be used, he returned to England, and soon afterwards joined E. O. Lawrence at Berkeley in work on the electromagnetic isotope separation for the atom bomb. Lawrence was another colleague to whom Oliphant had great affinity, and their relationship is also brought out very vividly here. The authors Colleges of Chence of Colleges of Chence of St. A P.C. No. Call & Like na P.Y. COARSLEX

Department of :
Chemical Engineering
Chemical Technology
Plants & Rubber Technology

describe the shock Oliphant felt when he heard of the use of the atom bomb on Japan, and his determination to work for solutions that would make future atomic war impossible or at least unlikely.

Back to academic life, to complete the construction of the cyclotron started before the War, and to start the proton synchrotron, based on an idea proposed by him but published by others first. The construction of two machines in a very confined space, and in a Britain beset by post-War shortages, using do-it-yourself methods as far as possible, was a typical Oliphant act of courage and optimism. The less ambitious of the machines, the cyclotron, gave little trouble; the proton synchrotron took much longer to complete than had been hoped for, and was ready only long after the similar, though somewhat larger, "cosmotron" at Brookhaven.

In fact the machine was completed only after Oliphant had left for his next assignment, the Australian National University at Canberra. The book describes the part he played in building up the spirit of the new institution, with all the struggle that involved, and also his choice of a machine for the physics department, a 10 GeV synchrotron powered by a homopolar generator. Looking back, this was clearly an error of judgement. He was again over-optimistic about the prospect and the time scale. If the machine had been completed as planned, it would have been by some years the first to reach this energy range, though even then the slow repetition rate would have made it difficult to use. As it is, only the homopolar generator was completed, but it will not drive a synchrotron and has instead found application in work on plasma physics. In spite of this disappointment, he succeeded in creating a physics department with many activities in different fields, and a staff of high quality.

Last, but not least, comes his term as Governor, which I have already mentioned. This again is described with warmth, though without glossing over some errors and embarrassments.

Taken as a whole, the book is perhaps a little longer than necessary. But it is a pleasure to read, and it does justice to a great character, whose integrity, frankness and directness set an example to us all.

Sir Rudolf Peierls was with Oliphant in Cambridge from 1935 to 1937, and in Birmingham from 1937 to Oliphant's departure for Australia. He moved to Oxford in 1963, where he is now Emeritus Wykeham Professor.

Membranes layer by layer

Clive Ellory

New Comprehensive Biochemistry, Vols I and II. Vol.I, Membrane Structure. Edited by J. B. Finean and R. H. Michell. Pp.271. ISBN 0-444-80304-1. (Elsevier/North-Holland Biomedical: 1981.) Dfl.140, \$59.50. Vol.II, Membrane Transport. Edited by S. L. Bonting and J. J. H. H. M. de Pont. Pp.362. ISBN 0-444-80307-6. (Elsevier/North-Holland Biomedical: 1981.) Dfl.140, \$59.50.

MEMBRANE expansion is a term that not only applies to certain important cellular processes, but also describes the status of the whole field of membrane biology. In addition, it seems to have become a clarion call to publishers. Predictably, however, books on the topic are extremely variable in quality, often representing unrefereed reportage at symposia. Happily, the present two works are excellent, comprising comprehensive and scholarly reviews by experts, each of whom describes the present state of knowledge in his area of membrane biology.

The depth in which subjects are covered in the first volume, Membrane Structure, is impressive, and is reflected by the relatively few (six) chapters of which it is composed. An initial contribution by the editors sets the scene and introduces the Singer-Nicholson fluid mosaic model - now ten years old and very much part of the established orthodoxy — as probably the last generalizable membrane model. They suggest new models will not be general, instead emphasizing the individuality of specific membranes at an advanced level of definition. Edidin produces a detailed and critical analysis of the results obtained from applying spectroscopic techniques to measuring molecular motions of membrane components, particularly the lateral diffusion of membrane proteins, op den Kamp reviews the evidence for asymmetry of both membrane proteins and lipids. Although agreeing that membrane proteins are always distributed in a completely asymmetric manner, he ends on a cautious note with regard to phospholipid asymmetry, counselling care in the interpretation of experimental results which rely on phospholipid modification during localization.

In his excellent review of membrane glycoproteins, Gahmberg starts by admitting that we do not know the reasons why all known cell surface proteins are glycoproteins. He finishes, however, on a more positive note by proposing two plausible roles for the carbohydrates attached to surface proteins.

The final two chapters deal with the demanding topics of membrane-bound enzymes (including the complexities of the adenyl cyclase system) and the structure and assembly of membrane proteins. Again the overall quality is impressive,

although some results are perhaps presented more optimistically than they deserve; for example, Freeman's discussion of Arrhenius plots could have included a word of caution on kinetic artefacts.

The credentials of the contributors to Vol.II, *Membrane Transport*, are equally impressive, but the topics are narrower or at least their treatment is less comprehensive. This is reflected numerically with 12 chapters instead of 6. Nevertheless, every contribution is worthwhile, and I particularly liked the review of anion-sensitive ATPase(s) by de Pont and Bonting, which raises the status of this system by focusing attention on the amount of information now available. In contrast, the contribution on SR Ca-ATPase seemed surprisingly lacking in data on structure and subunit composition.

The reviews on membrane permeability

for water and polar and lipophilic molecules by Sha'afi and Stein respectively, and the contribution by Weinstein et al. on the coupled transport of water reveal that we have made less progress towards understanding transport in membranes than we have towards elucidating their structure.

Stein's major contribution to Vol.II, on the kinetics of mediated transport, is presented with his usual rigour and vigour, and may have the consequence of promoting an uneasy conscience in those of us who publish papers defining membrane transport systems with less than the full kinetic treatment.

Technically the quality of printing and illustrations is good, and these are books which few membrane biologists can afford to be without. I hope this series can maintain its momentum and adhere to the overall title of the New *Comprehensive* Biochemistry.

Clive Ellory is in the Physiology Department, University of Cambridge.

Evolution: opinions, axioms and "isms"

Jonathan Howard

Evolution: Genesis and Revelations, with Readings from Empedocles to Wilson. By C. Leon Harris. Pp.339. Hbk ISBN 0-87395-486-6; pbk ISBN 0-87395-487-4. (State University of New York Press: 1981.) Hbk \$29.50, £20.65; pbk \$9.95, £7. Science, Ideology, and World View: Essays in the History of Evolutionary Ideas. By John C. Greene. Pp.202. Hbk ISBN 0-520-04217-4; pbk ISBN 0-520-04218-2. (University of California Press: 1981.) Hbk \$16.50, £11.50; pbk \$5.95, £4.

DESPITE a respectable suit of clothes, Darwinism remains deeply controversial. Even intricate internal disputes, over cladistics, over sociobiology, spill over into the public domain in extraordinary displays of bad temper in the popular press (if I may so designate, in this context, *The Times*). It is quite funny how many people feel entitled to express a public opinion regarding, say, the validity of the theory of evolution, from a standpoint of the most abysmal ignorance of any biological phenomena at all save the workings of their own bodies.

Controversy, if raucous enough, takes the reading public to the bookshops, only to be confronted with the mountain of Darwin and evolution studies. Nothing is more intellectually stultifying than to be faced with too much to read, yet more and more stuff is pressed on our attention. If, as seems likely, the evolution debate in the wide sense marks one of the most farreaching intellectual revolutions in history, it would be a pity to devote what little time

one has to reading the wrong books. If the publishers refuse to discriminate between worthwhile and trivial books, the critics must.

Here is such a discrimination. I have two review copies of books on the history of evolutionary ideas, one a flabby paperback by a (to me) unknown biologist, the other an elegant hardback by a distinguished historian of biology. But if the general reader based his judgement on these superficial observations, and settled for the elegant book by the distinguished author, he would make a grave mistake. For it is not worth his attention, while the less favoured volume is brilliant and original, and still more exceptional in being very funny. Evolution: Genesis and Revelations, with Readings from Empedocles to Wilson is the punning, bathetic title of C. Leon Harris's primer in the history of evolutionary ideas. Brief readings from the evolutionary masters are sandwiched between essays by Harris in which he first introduces, and later discusses, his readings for the session. The great joy of the book is that it is deeply opinionated. There is nothing more agreeable than finding a scholar with a clear point of view, clearly stated, and supported with a will and a wit. Harris has all of this. His discursive essays cover an extraordinary range, each one a gem of concentrated opinion and argument which flows onto the page at a terrific pace. Sometimes, indeed, the pace seems too great for the constraints imposed by English grammar, but after reading a few pages I could

forgive Harris even that. The first few chapters of Genesis (in a most bizarre translation; could it be a Gideon Bible?) provoke a savage attack on the intellectual folly of modern creationism:

The idea of equal time for alternative theories is seductive to fair-minded people, but if creation is not scientifically supported it has no more right to equal time than the stork theory of childbirth.

Excerpts from Augustine and Aquinas bring an essay on the power of prejudice constructed around McCarthyism and Close Encounters of the Third Kind. Readings from Diderot, Maupertuis and Lamarck are followed by an essay on visual illusion as a metaphor for scientific creativity. Having planted his idea, Harris is happy to point to its limitations:

... visual illusions, though interesting and perhaps useful as models of creativity, are not as emotionally satisfying as the real thing. No scientist who has lost sleep because of the thrill of creating his own theory will underestimate the power of inspiration. We must go to another model to study this emotional component of creativity. One such model for the creative scientist happens to be a chimpanzee named Sultan, who one day discovered that he could connect two sticks and rake a banana into his cage. He got so excited over his creativity that he forgot to eat the banana.

It would be wrong to leave the impression of Evolution: Genesis and Revelations as a collection of quick-fire one-liners. Harris is funny and original in his approach, but he is also extremely thoughtful nearly all the time. Unexpectedly, however, he makes extraordinarily heavy weather of his discussion of Darwinian evolution, following readings from Darwin and Wallace. It is here, if anywhere, that one might hope that so clear and refringent a mind would throw new light, or at least make a new joke. Well, we get the new joke, in the shape of "Harris's First Law of Knowledge: Belief in the truth of a theory is inversely proportional to the precision of the science", but no new light. Indeed I am prepared to match Harris's trenchant style and say that I think his claim of axiomatic status for Herbert Spencer's phrase "survival of the fittest" is misleading at best, and at worst, wrong. I cannot accept that a noun phrase can ever be an axiom. 'Survival of the fittest' is no more an axiom than "C. Leon Harris". Let us suppose that Harris intends "the fittest survive" every time he says "the survival of the fittest". Does this truism indeed lie at the root of Darwinism, and is it really necessary to assimilate it as an axiom to appreciate the quality of Darwin's thought? Certainly not. The phrase, and its axiomatic form, are sloppy portmanteaux. The foundations of Darwinism are generalizations from experience and lend themselves easily to falsification. They are acceptable because they have not yet been falsified. To put the generalizations simply, there is heritable variation between

individuals, and individual reproductive performance is not independent of individual variation in other characteristics. Not as catchy as "survival of the fittest", but at least nearly right.

If "survival of the fittest" can lead so acute a mind as C. Leon Harris's into folly, we should perhaps learn to avoid the coiner of the phrase, in case he should disguise any more complex truths as simple falsehoods. I propose that books on the history of evolutionary ideas are to be avoided in direct proportion to the number of references they make to Herbert Spencer. Using this criterion, we should certainly select Harris, with not a single reference. and A.O. Lovejoy's classic The Great Chain of Being. By the same lights we should unhesitatingly reject John C. Greene's Science, Ideology, and World View, the second of my two books. Greene devotes almost as much space to Spencer as to Darwin, even coining the unhappy term Spencerianism-Darwinism to encapsulate his sense of a zeitgeist. Spencer had a total inability to make himself clear. He also wrote at inordinate length. (Publishers were more responsible in the nineteenth century: one of the most important facts about Herbert Spencer is that he was obliged to publish the ten monstrous volumes of the Synthetic Philosophy at his own expense). It is no surprise, therefore, that the working of Herbert Spencer's thin ore had to wait until the mother lodes had been worked out. If C. Leon Harris lives in a world of bright lights and dark shadows, John C. Greene lives in a penumbra of obscure "isms" (a horrific word which actually introduces one of his essays). In a parody of scholarship, Greene includes part of some correspondence between himself and Ernst Mayr, concerning Greene's doomed attempt to confine the use of the word "Darwinism" to a package of threadbare Victorian prejudices. As Mayr rightly says,

You may make a few sociologists happy by inflicting that package of Spencerian concepts upon Darwin, but those who probably use the word Darwinism the most will simply ignore it.

Where I have the greatest sympathy for John C. Greene's book is at the end of his last essay where he appeals with a gentle candour on behalf of the moral imagination of man against the spiritless encroachments of prescriptive sociobiology. Harris, too, ends his book with prescriptive sociobiology, but with characteristic combativeness he uses a muddy piece of Wilsonian gobbledygook as an excuse for whipping "the Marxist opponents of biological determinism". This may be another elaborate C. Leon Harris joke, but it is subtly done and for once I cannot see even a hint of a smile.

Jonathan Howard is at the ARC Institute of Animal Physiology, Babraham, Cambridge, and author of Darwin, recently published by Oxford University Press.

Scattering wisdom

Roger Pynn

Pulsed Neutron Scattering. By C.G. Windsor. Pp.432. UK ISBN 0-85066-195-1; US ISBN 0-470-27131-0. (Taylor & Francis/Halsted: 1981.) £25, \$85.

A GENERATION ago, a mutually beneficial relationship between solid-state physicists and the growing community of reactor specialists resulted in the birth of neutron scattering. Subsequently this field has grown to attract scientists from many other disciplines; chemists, biologists, metallurgists and even archaeologists have profited from the ever-increasing sophistication of neutron spectrometers.

Now, as reactor-based facilities are nearing their maximum performance, a new symbiotic relationship is beginning as neutron scatterers take over unfunded particle accelerators to produce pulsed neutron sources. This book describes such sources and the spectrometers which will use them. Although the book will probably be used principally for reference, the writing is sufficiently fluent that it can be read from cover to cover without undue stress. Indeed, the reader who does so will be rewarded by the occasional throw-away line incorporating practical wisdom in a pleasantly unpretentious style. The author has avoided irrelevant detail and the explanations are generally easy to follow. This is in no small measure the result of an excellent use of figures to explain the physical conformation of assorted hardware, as a guide through reciprocal space and to present illustrative experimental data. Mathematics, too, has been used intelligently. Where a mathematical derivation will be instructive, it is provided; otherwise results are usually quoted with sufficient reference to original work to allow the enthusiastic reader to find the derivations if he should need them. The simple, but usually neglected, expedient of "putting the numbers in" is frequently used to good effect, leaving the reader with a feeling for quantities as disparate as energy-resolution and moderator size.

As well as being an excellent compendium of much currently accepted wisdom, the book contains a number of contributions which are new. For example, detailed comparison of the performance of reactor and pulsed-source spectrometers has not previously reached a wide audience. Although not uncontentious, Dr Windsor's contribution in this field will certainly quell much argument — a curious effect often observed when fact is injected into a debate. The book also provides a

Academic Press have issued a condensed edition of Daniel Hillel's twin volumes, Fundamentals... and Applications of Soil Physics (for review see Nature 295, 466, 1982). The new book, Introduction to Soil Physics, costs \$22.50, £14.50 in paperback.

basic practical guide indispensable to anyone attempting an experiment with a pulsed-neutron spectrometer; in fact the numerous general remarks about experimental technique and spectrometer design are equally useful for the reactor-based scientist and the book should be compulsory reading matter for any new graduate student in the field. It is refreshing to find a discussion of the health hazards associated with working in weak irradiating fields; one wonders why a subject of such enormous potential importance has escaped attention in previous treatises on neutron scattering.

Apart from trivial typographical errors, I can fault the book only on two minor scores. In my view the first chapter does not do justice to its title of "Beyond Thermal Neutrons". I was expecting to be told of new science to be studied but read only the standard liturgy on the usefulness of neutron scattering. The chapter is really a thumbnail sketch of classical problems in neutron scattering and should have been titled as such. My second, less serious, objection concerns the partial use of SI units (for lengths only apparently). This is insufficient to satisfy the pedant but is more than enough to be irksome to those schooled in the conventional usage.

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Relating to halogenated hydrocarbons

Alastair Hay

Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. Topics in Environmental Health, Vol. 4. Edited by R.D. Kimbrough. Pp.406. ISBN 0-444-80253-3. (Elsevier/North-Holland Biomedical: 1981.) \$95, Dfl. 195.

TEN years ago the only environmental pollutant people were familiar with was DDT. Today you could list dozens of additional names. Two types of chemicals which would be included are the polychlorinated biphenyls (PCBs) and the polychlorinated dibenzodioxins (PCDDs).

Both the PCBs and the PCDDs, known collectively as chlorinated hydrocarbons, have been widely discussed, but the halogenated isomers of these compounds are not as well known. This new book on halogenated hydrocarbons will remedy this situation. Here, for the first time in a single volume, the properties of the PCBs and PCDDs are considered in relation to the whole family of halogenated hydrocarbons.

In the case of the PCBs there is some irony in the fact that the very physical properties which made these chemicals so popular are the reasons why they are so much of a problem today. The attributes of the PCBs were said to include thermal stability and resistance to both oxidation and chemical degradation. An estimated 1 million tons have been produced since 1930.

Numerous surveys have shown that the residues of PCBs can be detected in human beings. The problem appears to have been caused by eating food — and fish in particular — contaminated with PCBs, simply because there is such an abundance of these chemicals in the environment. Once these facts were known the bottom dropped out of the PCB market. Government regulations — long overdue — helped to hasten their demise. But it was only production which was affected; we still have the mess to clean up.

It is a different problem with the PCDDs, which are produced as unwanted by-products in the course of making chlorinated phenols. Often they remain as contaminants in the finished phenolic product, some of which are widely used. Pentachlorophenol, for example, is a common wood preservative. And 2,4,5-trichlorophenol — contaminated with the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) — is the starting point for the manufacture of the herbicide 2,4,5-T.

Although the threat from environmental exposure to both PCBs and PCDDs is still unknown, there is much more evidence about the hazard to workers occupationally exposed to them. It emerges that, as far as toxicity is concerned, there are

many similarities between the two substances.

In her introduction to this book, Renate Kimbrough says that it was thought to be desirable to discuss PCBs, PCDDs and related compounds in one book to help convey "a better understanding of their action and the complexities of their toxic effects".

The book does just that and it does it very well. Kimbrough has persuaded leading scientists in the field to discuss the chemicals in depth and to draw comparisons between them wherever possible.

The subjects included cover everything from the chemistry of these chemicals to their production, usage, and animal and human toxicology. In addition, there are valuable case studies of human populations exposed to PCBs in Japan and to TCDD at Seveso in Italy. The last chapter, by the editor herself, discusses the problems and health effects of occupational exposure to such substances.

The book is likely to become one of the standard texts for both students and researchers concerned about the health effects of exposure to PCBs, dioxins and their ilk. It is a joy to read a book as well edited as this, where there is no overlap between chapters and where all the contributors have stuck to their brief. The reader is left in no doubt that the toxicity of these halogenated aromatic compounds is indeed complex, but that there are many similarities between such chemicals in the way they exert their toxic effects.

Alastair Hay is a Lecturer in the Department of Chemical Pathology at the University of Leeds.

Foram stratigraphy

F.T. Banner

Stratigraphical Atlas of Fossil Foraminifera. Edited by D.G. Jenkins and J.W. Murray. Pp.310. UK ISBN 0-85312-210-5; US ISBN 0-470-27191-4. (Ellis Horwood/Halsted: 1981.) £25, \$79.95.

IN ONE concise yet comprehensive handbook, the value of fossil foraminifera to the stratigraphical geology of the British Isles is demonstrated at last. Before this publication, the information was scattered and no clear over-view could readily be obtained. Now, a collaboration by 23 authors (drawn from the petroleum industry, industrial consultancies, universities and the Institute of Geological Sciences) has provided a summary of all useful, published information, and careful

editing has presented it systematically and intelligibly.

Each of the 12 chapters outlines the stratigraphical base for the micropalaeontology of each successive geological interval. The foraminiferal record of the Pre-Carboniferous is poor, but in succeeding strata the value of foraminifera to their biostratigraphy becomes very apparent. The seven stages which comprise the British Carboniferous succession may be recognized and divided on the distribution of foraminiferal genera alone. The following chapters on the Permian, Triassic, Jurassic, Cretaceous, Palaeogene, Neogene and Quaternary intervals demonstrate how the stages, macrofossil zones (where they exist) and principal lithostratigraphic units are characterized by foraminiferal genera or species and by their assemblages. One short chapter summarizes the available information on the North Sea Cenozoic

In all, 570 important foraminiferal taxa are illustrated (usually by scanning electron microscopy, but, where hard limestone lithofacies predominate, by optical thin sections), and for each the essential taxonomy and morphology are outlined and the source of the illustrated specimen is noted. Where it is possible to do so, stratigraphic ranges (as syntheses or as objectively recorded occurrences) are illustrated by graphs. Each chapter on stratigraphy also records the whereabouts of the more important reference collections. Where it can be attempted, there are discussions on palaeoenvironmental and palaeofacies influences stratigraphical record; the last short chapter outlines the successions of foraminiferal assemblages recognized through the whole Phanerozoic, as a product of both evolution and of changing environments.

This is a most useful book; it will become a standard reference for all stratigraphic geologists and micropalaeontologists concerned with the post-Devonian strata of the British Isles. The format is clear, convenient for reference and methodical; the printing of text, diagrams and photographs is to a high standard. Some users will argue with the nomenclature which has been employed, and others will fault the taxonomy, but this is inevitable. More serious criticisms are that the essentially provincial scope of the book is not indicated by its title, and, in some chapters more than others, the welter of local British place-names (not adequately clarified by location maps) will confuse the overseas reader. This said, the editors, authors, publishers and the British Micropalaeontological Society are to be congratulated on this concise, attractive and much-needed volume.

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Rules for the environmental game

David Dickson

DDT: Scientists, Citizens and Public Policy. By Thomas R. Dunlap. Pp.318. ISBN 0-691-04680-8. (Princeton University Press: 1981.) \$24.50, £13.10. The Reserve Mining Controversy: Science, Technology, and Environmental Quality. By Robert V. Bartlett. Pp.293. ISBN 0-253-14556-2. (Indiana University Press: 1981.) \$17.50, £10.50.

"I've never seen a company, even among the most enlightened, that considered environmental affairs to be anything more than a public relations problem" one of the three members of President Carter's Council on Environmental Quality said shortly after returning to his previous role as an environmental activist earlier this year.

The charge is far from one-sided. In an important sense, the various environmental debates that have taken place in the United States over the past 20 years have, indeed, been part of a large public relations game. Refusing to believe that a serious problem exists, many private companies have been more concerned with demonstrating the legitimacy of their existing practices than with volunteering the need for stronger federal restrictions. For its own part, the environmental movement recognized at an early stage the need to operate in a highly-visible public arena. Close relations with the mass media, reinforced by a well-developed sense of what makes a good news story, helped generate a mostly-sympathetic press, which in its turn served to mobilize public support.

Behind the public relations front, of course, more serious issues have been fought out. The growth of the environmental movement in the early 1960s was one of the first significant challenges to traditional mechanisms for the control of both industrial technology and its sideeffects. Previously, discussion about the environmental impact of new industrial or agricultural practices was contained largely within professional circles of scientists and physicians. The public had little cause - or inclination - to question the views of those it accepted as experts. The environmental movement openly challenged this mould. What started as a few isolated protests soon snowballed, in the face of apparent official indifference, into a nation-wide bandwagon on which many politicians were quick to jump. In the process, channels for public participation were opened up that significantly increased the opportunities for non-professional groups to intervene in the process of determining what technical innovations were and were not socially desirable. The most important development was the National Environmental Protection Act of 1969. By permitting environmentalist

groups to question the scope and adequacy of "impact statements" required for any major federally-funded project, NEPA provided a platform from which challenges to a wide range of government initiatives could be launched.

Even before NEPA, however, the environmentalist movement had found that, if public relations was its most effective political weapon, the courts were its most advantageous battle-ground. The courts were relatively neutral territory where environmentalists could set their own agenda and argue on equal terms with government administrators and industrial manufacturers alike. No where were the benefits of this strategy more clearly demonstrated than in the campaign which eventually led to the decision of the Environmental Protection Agency to ban the use of DDT in 1972.

Production of DDT in the United States had risen to about three million pounds a month by the end of the War, and shortly afterwards it entered civilian use with a reputation of being such a miracle chemical that one entomologist speculated that it might eventually banish all insect-borne diseases from the Earth. Yet as its use to protect agricultural crops, promoted by government scientists, grew rapidly, the first reports of mortality among birds and fishes began to be heard from wildlife biologists, indicating that DDT might not be the complete miracle that it appeared.

The early reports were not ignored. But neither, as Thomas Dunlap demonstrates in his closely-researched history of the campaign against the pesticide, DDT: Scientists, Citizens and Public Policy, were they initially taken very seriously. To farmers and pesticide manufacturers, the death of a few robins was a small price to pay for the benefits that the pesticide had brought.

The turning-point came with the publication of Rachel Carson's Silent Spring in 1962. Mrs Carson presented the various data that biologists had collected showing the different effects on wildlife that DDT seemed to be having, in particular how it was being concentrated through the food chain. But she went further, using the raw data to build an indictment of modern industry that soon became a battle-cry for the environmentalist movement, turning many conservationists into political activists.

If it took only one individual to open up the issue, more effort was needed to secure political change. Much of Dunlap's book is taken up with a close description of the case against DDT that was presented in 1968 by the newly-formed Environmental Defense Fund against the state of Wisconsin. Selecting its legal ground carefully, the EDF was granted a legal hearing to evaluate its claim that the scientific

evidence against DDT, for instance its link with declining bird populations such as that of the peregrine falcon, was sufficient to have the chemical declared an environmental pollutant for the purposes of regulation.

The EDF won its case. A sympathetic judge agreed that the evidence against DDT was sufficiently damning, and that there was no identifiable level beneath which DDT could be claimed to be completely harmless, a conclusion which undoubtedly contributed to the EPA's decision to phase out the chemical in 1972. Yet as much as the verdict itself, it was the publicity generated by the court case that provided some of the most effective spinoff. For it helped to generate a wave of public support for strict environmental laws — such as NEPA — that sympathetic legislators were quick to pass through Congress.

Industry, too, learnt its lesson from the Wisconsin hearing. The National Agricultural Chemicals Association initially sent only one retired attorney, a former counsel for the Velsicol Chemical Corporation, to present its defence of DDT. Having little reason to doubt that the arguments about the lack of evidence of DDT's harmfulness, which had proved successful in previous government regulatory proceedings, would again prevail, he was unprepared for either the style or the strength of the environmentalists' carefully-prepared attack. Even if it was a public relations battle, the symbolism of the loss in Wisconsin had to be taken seriously — a message which the chemical industry gradually took to heart during the 1970s.

The path seen in the struggle against DDT, culminating in hearings before the Environmental Protection Agency in 1971 and its eventual ban by EPA administrator William D. Ruckelshaus, was soon to become familiar. A similar process led to the ultimately-successful efforts by residents of Wisconsin's neighbouring state, Minnesota, to stop the Reserve Mining Company from discharging waste tailings from its taconite mill directly into Lake Superior, the largest inland sea in the Western Hemisphere.

In The Reserve Mining Controversy: Science, Technology and Environmental Quality, Robert V. Bartlett describes how the final outcome of that campaign, fought in various state and federal courts over a period of more than ten years, was as much the result of changing public perceptions as a neutral assessment of scientific evidence that the tailings discharged into the lake were causing harm.

The parallels with the DDT campaign are close. Just as the first critics of DDT based their case on the environmental effects of the pesticide which much of public opinion could dismiss as relatively harmless, so the first complaints against Reserve Mining, which had been given permission to discharge its tailings into the

lake when building its plant in 1947, focused on the discoloration of the water that the discharge appeared to cause, even though there was no direct evidence that the discoloration was causing significant biological damage.

Similarly, just as the final nail in DDT's coffin was the discovery early in 1969 that the chemical was a potential human carcinogen, so the federal order requiring Reserve Mining to find a land-based depository for its tailings — the result of a drawn-out suit against the company filed by the Department of Justice at the instigation of the EPA — hinged on the close morphological similarity between particles in the taconite tailings and asbestos fibres which scientists were beginning to demonstrate posed a significant hazard to health.

Bartlett, like Dunlap, provides a useful account of the twists and turns of the legal proceedings. The Reserve Mining controversy provides, as he points out, perhaps one of the most dramatic examples of the difficulties facing courts that are asked to decide on relatively complex scientific issues with legal equipment often ill-suited to the task. Each side in the dispute exploited the ambiguity of evidence about the environmental and health effects of the taconite waste to its own advantage, applying criteria of "truth" and "legitimacy" entirely foreign to conventional scientific discourse. As Bartlett points out, the dilemmas faced by the courts on such issues are partly the result of built-in tendencies of the political system in the United States to try to resolve conflict through adversary litigation rather than consensus-based planning. Dunlap, however, goes further to argue that the environmental problems raised by persistent pesticides such as DDT are not merely the result of unthinking industrial growth, or shifting public perceptions, but have deeper social and political roots. Among these, he suggests, is a "technicalfix" mentality to pest control that was reinforced by the professional self-image of economic entomologists, physicians' concepts of health and disease, and the economic motivations of farmers and pesticide manufacturers.

The significance of this message is unlikely to disappear. The climate of public opinion has changed since the days when the environmentalist movement was at its height, yet the change may not be as dramatic as many in the Reagan administration would like to believe. Plans to overhaul the Clean Air Act, for example, offered as a pre-election promise to the auto industry in 1980, have been postponed largely on the grounds that the political price in terms of lost popular support would be too high. Rather than abandoning the ideal of environmental protection, the administration is seeking more subtly to change the ground-rules.

The lesson of both of these books is the need to treat semantics warily. In both the DDT and the Reserve Mining disputes, the struggle over the proper treatment of the natural environment became clothed, for legal purposes, in a scientific language that as often seemed to obscure as clarify the real nature of the conflict. The problem is not unique to the United States, but it tends to be exacerbated in a country where, as one social scientist recently put it, "the gap between political ideal and political reality is a continuing central phenomenon".

David Dickson was Washington News Editor for Nature from 1977 to 1982.

AI in chemistry

R. Venkataraghavan

Applications of Artificial Intelligence for Organic Chemistry: The Dendral Project. By Robert K. Lindsay et al. Pp.194. ISBN 0-07-037895-9. (McGraw-Hill: 1981.) \$39.50, £19.95.

Applications of Artificial Intelligence for Organic Chemistry provides an excellent overview of a discipline that is playing an increasingly important role in problemsolving techniques using computers. The volume takes the reader through the definition of a problem in organic chemistry, concepts of advanced computer techniques, applications and, finally, to some interesting observations and conclusions of the authors. The applications cited in the book represent the research carried out by a team of interdisciplinary scientists, and provide some unusual and thoughtprovoking perspectives on the promise of computing in this area.

Emphasis is placed on the utilization of spectrochemical data and Dendritic Algorithms for structure-elucidation problems. The evolution of the Dendral project and its current capabilities clearly show the potential of computer-based techniques in the symbolic manipulation of complex information. The application of these techniques, however, is not confined to organic chemistry; in time they will undoubtedly be used to tackle a number of other problems in applied science. Certainly, the discussions of design considerations and the choice of computer-based tools should benefit those readers involved in the development of a variety of complex software projects.

This concise volume, representing the fruits of several years of active research, will prove to be a valuable source book for all seriously interested in the applications of computer techniques to problems in organic chemistry. It is to be hoped, too, that its influence will spread to associated disciplines and beyond.

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nature

22 April 1982

Zeal on secrecy could backfire

Admiral Bobby Imman of the CIA knows the danger that his crusade to alert US researchers to Soviet espionage could make academics abreact. But what else is the Administration looking for?

The Reagan Administration may be heading for a confrontation with US universities reminiscent of the Vietnam era. The issue is the tradeoff between national security and academic freedom in the research universities and the charge by Administration officials that, if something is not done, US campuses will become the targets of intensified Soviet intelligence efforts to gain advanced knowledge in genetic engineering, very high speed integrated circuits, lasers and the like. So far, the dialogue between the Administration and several spokesmen for university science has been restrained, even cordial. But there is already some hardening of positions on both sides, and some Administration ire that the scientists are not cooperating, as well as some campus resentment of the calls for self-policing.

Some of this resentment surfaced on 29 March when two subcommittees of the House Science and Technology Committee of the
US Congress held a joint hearing to gather the Administration's view of
how the free flow of research threatens national security. The star
witness was Admiral Bobby R. Inman, the deputy director of the
Central Intelligence Agency (CIA). Inman was formerly director of the
National Security Agency (NSA), the government's secret code
agency, where he provoked a dialogue with university mathematicians
on the dangers of publishing advances in cryptography — and even got
some to submit papers voluntarily for government censorship. In
January of this year, Inman caused a stir when he ventured to the
annual meeting of the American Association for the Advancement of
Science and suggested that unless scientists themselves devised
mechanisms to prevent leaks to the Soviet Union, the government
might require them to submit their research to censorship before
publication.

At the House hearing in March, Inman elaborated his view of Soviet technical espionage in the United States, US counter-espionage and the campuses. At present, he said, approximately 70 per cent of Soviet technical intelligence comes through purchases of hardware - some legal, some illegal. Moreover, the Soviet Union does "a very thorough vacuum cleaning of anything in the public sector". Finally, there is a smaller effort, which has received special emphasis since the late 1970s, aimed at aquisition of technologies emerging from universities and research centres. US counter-espionage is aiming to cut off as much of the hardware aquisition as possible. So, Inman predicts, US campuses will become the focus of a much intensive espionage. In 6 months, a year or 18 months, he warned at the hearing, it will become clear to everyone, including the Administration, how much the Soviet Union is benefitting from the "outflow" of information from the campuses on some sensitive topics. Pressure will then grow in the Administration to crack down on universities - unless by then, they themselves have controls in place.

Inman's role in this crusade is odd. In March, he repeated that he was not representing the CIA or the Administration and that his testimony had not been coordinated with the other Administration witnesses. He described himself as a self-appointed "goad" or "gadfly" to get people to think about the problem and to suggest "innovating ideas" for cutting down the "outflow" from their laboratories to the Soviet Union. The trouble with his case, so far, is that few university scientists seem convinced there is a large outflow or "haemorrhaging" of information about very high speed integrated circuits, robotics or anything else. Inman admits his case rests on evidence contained in a classified report that cannot be released without compromising intelligence sources. But representative Albert Gore Jr (Democrat, Tennessee), chairman of the committee's sub-committee on

investigations and oversight, who has seen the classified report, ventured at the hearing that the full evidence is not all that convincing. Inman himself conceded that "In an open society, there is a tendency to automatically (sic) take the view that one cannot debate the substance of issues unless you see the evidence." He and the Administration should understand that the discussion of what the universities should do cannot begin until the evidence is on the table.

The Administration's new-found zeal for secrecy about academic research has already had some tangible consequences. Some months ago, on the eve of a visit planned for a Soviet robotics expert to the United States, the Administration asked Stanford University and some other places to close their electronics laboratories to him. The universities replied that they could not police their visitors, whereupon the would-be visitor was not issued with a visa. The truth is that US universities cannot easily act as espionage policemen, especially when they are trying to forge links with industry to give US industry an "outflow" of

Visitor: Here's \$250,000 to spend. No strings. Just let me be the first to know.

Scientist: Hey! Don't you have relatives in Eastern Europe? How do I know you're not a spy?

Visitor: That's none of your business. Anyway, I'd been thinking of making a deal with that competitor of yours in Berkeley. How do I get out of here?

research results it badly needs to stay competive with Western Europe and Japan. Does Inman really want US scientists to be suspicious that every foreign visitor or industrial benefactor may be a spy? (See box.) Can it be wise to breed such suspicions when university scientists are making their first fragile attempts to reach outside their laboratories?

In reality, however, the issues the Administration has raised with the campuses offer much middle ground and room for discussion. University scientists already live with a spectrum of controls — from peer review to federal regulations — that appear not to inhibit academic freedom. More and more people are learning about the new shadings of controls required when dealing closely with industry — the distinction, for example, between teaching only basic science in the classroom and keeping possible patentable discoveries off limits for a certain period. Moreover, the Administration is engaged on several reviews that could have enormous impact on university research. The Commerce Department is reviewing the International Traffic in Arms Regulations (ITAR), passed in the Cold War era of 1955 (and which is probably unconstitutional), that allows the government to prevent to the export, and possibly publication, of "technical data" - whatever that is. At the hearing in March, in chilling testimony, Assistant Secretary for Trade Administratrion in the department, Lawrence J. Brady, said that in the past, it has not been fully understood that ITAR applies to all segments of US society, including the universities. George Millburn, an undersecretary at the Department of Defense, testified that a "struggle" is going on in the department to define which technologies are militarily important and subject to review and control. Yet the only academic involvement in these discussions is some work by the Defense Science Board and a study by the National Research Council of the National Academy of Sciences

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(see page 695).

While there may be room for discussion, there are also the makings of a fight. Many academics or their champions, such as Gore, see in the Administration's words a threat to fundamental inquiry. Gore told Inman "we don't want to even (sic) take the first step along the road that has made Soviet science so pitiful". Inman, on his side, complained that the press and some academics misrepresented his statements to mean that he wanted to "throw a net over the public". Yet some universities have already taken avoiding action. At Stanford University the Faculty Senate has approved a resolution urging the university to resist interference with free scientific communication.

Academics are highly sensitive to any threats to their independence and tend to cry foul when anyone suggests they submit to any authority whatever — be it university administrators trying to enforce government accounting rules or suggested self-censorship of militarily important research. Moreover, President Reagan's military build-up and talk of nuclear war is stirring antagonism at many US campuses, as the recent wave of teach-ins and meetings attests. It would be all too easy for these issues to be rolled into one ball of wax in many academics' minds: burdensome federal regulations, arms build-up, nuclear war and government controls to prevent espionage. The resulting feeling would be that the Administration is against the universities and does not understand them — a feeling reminiscent of some campus attitudes towards former Presidents Johnson and Nixon. Given its penchant for loose, inflammatory rhetoric on other matters, the Administration may find it too easy to characterize campus resistence to controls as unpatriotic. The push from the right would then shove the campuses left. If the Administration keeps to Inman's timetable, the universities will have to step briskly—and calmly and rationally—to avoid an open conflict.

Big Brother's law

The British Government seems indifferent to its electors' needs for protection from data banks.

The British Government has simply failed to understand why there is public anxiety that computerized records may be a threat to people's privacy and, thus, liberty. That is the most charitable reading of the white paper on the subject, now at last published.* A more cynical, but probably more accurate, interpretation is that the government is squarely out of sympathy with the fear that computerized data banks may infringe the rights of individuals for which reason it will continue to drag its feet on legislation, doing everything it can to ensure that the eventual law is the bare minimum necessary to comply with the Council of Europe Convention signed a year ago.

The grudging character of the government's intentions can be told from the only statement in the white paper of the reasons why legislation is necessary. This mean document correctly records that the Council of Europe convention will require its signatories not to transmit personal data files to countries without satisfactory legislation, notes that this restriction could be a threat to international companies operating in Britain as well as to British computer bureaux and therefore concludes that "in order to conform with international standards of privacy protection and to avoid possible barriers to trade", the government will introduce legislation that will "enable the United Kingdom to ratify the convention".

Shabbily, nowhere is there a flicker of acknowledgement that the protection of people's privacy is in itself desirable, a public good of the kind which governments are elected to cultivate. Indeed, the government's sense that it owes something to its electors is expressed only in the repeated promise that the cost of administering the promised legislation will be kept to a minimum, and will be no charge on public funds. Even the salaries of the proposed registrar of personal data banks and his staff will be recovered from the fees that must be paid by their operators. Individuals seeking to verify the accuracy of personal data on

specific computer files will have to pay a fee "based on the principle that the costs for the demands are fully recovered". Those who, having paid their fees, think they have found their privacy to have been illegally infringed will have to mount prosecutions off their own bats, and at their own expense.

The government that happens historically to owe its existence to the Magna Carta, one of the earliest public declarations that tyranny is unacceptable to ordinary people, appears to be willing that ordinary people should take their chances in the courts if they have reason to believe that some injustice has been done by a data bank or those who operate it. Fortunately — this at least must be the hope — even the government's supporters in the House of Commons will be so affronted by what is now proposed that the unseemly legislation the government plans belatedly to introduce (not this year, perhaps not even next year) will be constructively and radically amended.

This is how libertarians in the House of Commons and elsewhere should argue. While computerized data banks are in principle no different from systems of records manually kept, they are potentially so much more efficient that their common use is tantamount to a qualititative change. So much has been acknowledged in the past decade by two government committees set up specifically to consider questions of privacy — the Younger committee (which reported in 1972) and the Lindop committee (reporting in 1978). False data, inadvertantly or even maliciously erroneous, can be damaging to those concerned. But even accurate data can be damaging, especially in the hands of those for whom they were not originally compiled. While it may be proper that information about people's dealings with tradespeople should be available to others in the same line of business, information such as this may be unwarrantably damaging in the hands of others, potential employers for example. Similarly, information about a person's medical history collected by one government department in the course of its legitimate business may be damaging when transferred to

So how should this transfer of data between different kinds of users be regulated? In its declaration of the principles on which the new legislation will be based, the British Government merely quotes the principles on which the European convention has been drawn (the chief of which is that a person should have access to any files about him, and the power to correct them if inaccurate or to erase them if illegally compiled). What needs to be decided, now and not later, is the basis on which the transfer of data may be allowed from one potlential user to another. It is simply not good enough to say that "it is expected that most applicants will be registered without question, but the Registrar will have power to make enquiries, to inspect data files and to require modifications to a system. In extreme cases, he may need to refuse registration. . .". What the government has so far failed to explain is how the registrar will decide which applications for registration are acceptable "without question".

The issue is especially important where the transfer of information between government departments is concerned. The European convention permits the exclusion from regulation of data banks "protecting state security, public safety, the monetary interests of the state or the suppression of criminal offences". This form of words is a sufficient licence for Big Brother. On the face of things, it will for example allow people's income-tax returns to be compared with their income from holdings of government securities or their applications for driving licenses to be checked against the records of the National Health Service, while the police will presumably be allowed to gather information from where they can in the cause of crime prevention. Are all these applications of data banks to be blithely sanctioned? By its repeated references to the European convention as the guiding principles on which the registrar will work, the British Government seems to intend just that. Parliament should insist that the European convention is not the final touchstone of what is permissible, but merely the starting point for what is bound to be a long and acrimonious argument.

*Data Protection, Cmnd 8539, 24pp. (HMSO, London, 1982) £2.30.

Oppenheimer case boils up again

Files show FBI bugged pre-trial conversations

Washington

A forthcoming history of science journal reveals new information on the celebrated Oppenheimer case — the most traumatic incident in post-war US science — that suggests that the government stacked the case against Oppenheimer in proceedings that caused the revocation of his security clearance and his personal disgrace.

Physicist J. Robert Oppenheimer led the Los Alamos atomic bomb project during the Second World War. Afterwards, he was the unquestioned leader among US physicists seeking to shape the post-war world. Oppenheimer was influential in the debate over civilian control of atomic energy and, later, chairman of the General Advisery Committee of the Atomic Energy Commission (AEC). Oppenheimer was then without doubt the government's most influential science adviser.

But doubts about his possible pre-war Communist associations, and his apparent evasions in relation to them, led AEC to conduct an inquiry into his suitability to hold security clearance, and to a hearing before a specially appointed triumverate of AEC's Personnel Security Board in 1954. The hearing, including the testimony of his one time colleague Edward Teller, destroyed him.

An article by Barton J. Bernstein, Professor of History at Stanford University, in Historical Studies in the Physical Sciences*, to be published later this spring, alleges that despite President Eisenhower's stated wish that the government should conduct the inquiry in a dignified manner, commensurate with Oppenheimer's stature, the Federal Bureau of Investigation (FBI) nonetheless bugged Oppenheimer's conversations with his attornies before the inquiry and passed the transcripts on to prosecutor Roger Robb. AEC chairman Lewis Strauss personally thanked the FBI for their help in supplying the transcripts. "It would be an uneven contest", even before the hearing, writes Bernstein, "just as if one army had stolen its enemy's secret plans".

Moreover, Bernstein says, while members of the board and the prosecution had access to secret FBI files on Oppenheimer (some of which were unopened at the time that the government had given Oppenheimer earlier security clearances), these were never shown to Oppenheimer or his attornies on the grounds that security clearance could not be obtained in time.

These and other revelations are based on documents declassified by the FBI, the Department of Energy (successor to AEC) and the Eisenhower Library at Bernstein's request. But Bernstein believes there is still more to learn about the case, as "more than half" of the FBI material on Oppenheimer remains classified.

The chairman of the three-member panel that finally judged Oppenheimer a security risk was Gordon Gray, former president of the University of North Carolina and former Secretary of the Army. Gray's papers, unearthed by Bernstein, show that Gray considered the other two members of the board to be completely prejudiced against Oppenheimer before the hearing even started. The three sat down together beforehand, to go through the files. Grav wrote of the other two, "It seemed to me from the beginning that Mr Morgan and Dr Evans both had a strong hunch that Dr Oppenheimer's clearance should not be reinstated . . . During the weeks spent reading through the files this notion became much clearer." Evans made anti-Semitic comments, saying that he found "that nearly all subversives were Jews". "At the time I was concerned at this note of clear prejudice", Gray wrote. But he did not tell AEC that the board's members were not impartial as AEC rules required.

Bernstein's new documents also reveal that Edward Teller, whose testimony was crucial, had told the FBI some years earlier of his doubts about Oppenheimer, and that Teller's statements helped shape the charges. Teller, however, has said that he was undecided about Oppenheimer until the night before he testified, when Robb showed him Oppenheimer's security record.

But in 1952, according to Bernstein's article, Teller told the FBI that he would do almost anything to separate Oppenheimer from the General Advisery Committee because Oppenheimer had cleverly shifted from one argument to another on the committee and used his considerable influence over other scientists to delay development of the hydrogen bomb. In 1952, Teller had not said that Oppenheimer was disloyal, only that he was dangerous because he gave bad advice. To Teller in 1952, Bernstein writes Oppenheimer "was devious but not disloyal". He had persuaded Hans Bethe to refuse to work on the H-bomb and had swayed Henry D. Smyth, an AEC commissioner, to oppose it.

Bernstein believes that his new information also provides an even more "alloyed" portrait of Oppenheimer himself than has emerged in previous writing about the case, which has tended to portray Oppenheimer as a martyr. The documents show, Bernstein writes, evidence of "a string of lies and evasions" regarding his past, and that Oppenheimer had not always been candid with the authorities or his associates. Commenting on the strain that Oppenheimer visibly underwent at the hearing, Bernstein writes:

Oppenheimer was struggling to preserve his reputation . . . He lost. And he may have been under an additional strain that his defenders did not appreciate, for he may also have been hiding his former membership in the Communist party. Chevalier claimed that Oppenheimer had been a member, and Robb reached the same conclusion.

However, except for wondering what is in the yet unopened files, Bernstein says there

Merck backs UK neuroscience

Dr Leslie Iversen, director of the Medical Research Council's neuro-chemical pharmacology unit at Cambridge for the past eleven years, has been appointed director of a new laboratory being established in Britain by Merck, Sharp and Dohme. The new laboratory, which will be a going concern on a greenfield site near Harlow, Essex, by 1984, will be exclusively concerned with basic drugoriented research in the neurosciences and will eventually employ 200 people, a third of them with research degrees.

The British scientific community is delighted at this development, chiefly because of the jobs it will provide for researchers but also because it is a token of continuing American interest in the United Kingdom as a base for major research laboratories.

Dr Iversen said earlier this week that he was looking forward to the job, but emphasized that while the new research centre would concentrate largely on basic neuroscience, it would be an integral part of the company's research effort and thus different from the Roche research centres in Switzerland and the United States. The hope, he said, was that basic neuroscience research would suggest novel classes of therapeutic drugs.

The only cloud on the horizon is the future of the Medical Research Council's unit, which is to be decided in June this year and which has been for some years one of the brightest feathers in the council's cap. Dr Iversen says that he is anxious that the unit should survive his departure on 1 March next year, for which reason he intends to avoid recruiting any of his present staff until the council has made up its mind. Apart from the unit's basic research, it also houses the United Kingdom "brain bank" from which authenticated samples of brain tissue are distributed to qualified investigators.

^{*}Historical Studies in the Physical Sciences, Office for the History of Science and Technology, 470 Stevens Hall, University of California, Berkeley, California 94220 USE

is no direct evidence in the new material that Oppenheimer had indeed been a Communist.

Deborah Shapley

Soviet radio-catastrophe

Another theory

Washington

What did happen in the southern Ural mountains in 1957–58? Did buried radioactive waste explode? Or was the landscape blighted and thousands of people hurt by a strange weather pattern that rained fallout from Soviet atomic bomb tests in the north?

The disaster has been a mystery since it surfaced in the West in a Copenhagen newspaper report in 1958 that implied that a Soviet weapons test had gone awry, and speculation grew into controversy in 1976 when Zhores Medvedev, a prominent Soviet dissident scientist living in London, alleged there had been a catastrophic, volcano-like explosion from buried nuclear wastes in a secret area near the city of Kyshtym.

Now a new theory has been advanced by the Los Alamos Scientific Laboratory, that links the disaster, among other things, with acid rain. The public summary of a classified report concludes that there was no single catastrophic explosion of buried wastes, and certainly not an explosion involving nuclear criticality. Instead, widespread damage to the land and population was caused by "a series of relatively minor incidents . . . severely compounded by a history of sloppy practices" at a plutonium production plant.

The new report confirms the allegations of Medvedev and Soviet scientist Lev Tumerman, who drove through the region in 1960, in their horrid accounts of burned people and devastated land. It recounts that there were "death squads" of radioactively contaminated prisoners assigned to work in the area, essentially until they dropped dead.

The site of the alleged disaster was a plutonium production plant built for the Soviet weapons programme east of the city of Kyshtym on the site of a munitions plant that operated under the Tsars. The plutonium plant was apparently built in the late 1940s, after the Smyth and Groves reports had been published explaining how the United States had built its atomic weapons during the war. The report indicates that the Soviet Kyshtym plant was modelled on the US plutonium plant at Hanford, Washington, and that, the Soviet Union had detailed information about Hanford practices and procedures. The evidence for this, however, is not given.

So, the report says, a city was built having "thousands" of workmen and engineers. A graphite-moderated, uranium-fuelled reactor was built, called "Unit O". Cooling water from Unit O was pumped into a large artificial lake dug on the grounds. Later, the cooling water from

Rorvik disowned

New York

Dr Derek Bromhall, formerly of the University of Oxford, has won his suit against journalist David Rorvik and the publisher Lippincott and Company, On the third day of the trial this month (see Nature 1 April, p.383) and before a single scientist had taken the witness stand, the defendants settled out of court. The settlement is important not only because it amounts to a public disavowal by the publishers of Rorvik's book, published in 1978 under the title In His Image: the Cloning of a Human, but also because it is a precedent for other complaints of the unauthorized use of scientific data in popular works.

In an apology to Dr Bromhall which accompanied the settlement (said to amount to some hundreds of thousands of dollars) Martin H. Lippincott, Chief Executive Officer of the publishers, conceded that "this company now believe the story to be untrue" acknowledged that "Dr Bromhall did not consent to the inclusion of his name or his research techniques in the book and also that Dr Bromhall never engaged in, or attempted to engage, or advocated the cloning of a human being. We regret any embarrassment, humiliation, or other injury to him that the reference to Dr Bromhall in the book might have caused".

Lippincott and Company had promoted the book, about an unnamed American millionaire financing the birth of his clone, as non-fiction.

Judge John Fullam of the United States District Court, who had been hearing Dr Bromhall's suit last year, called the book "a fraud and a hoax". Apparently Lippincott began withholding royalty cheques from Mr Rorvik as soon as the initial suit began.

As Dr Bromhall is no longer an academic scientist, the direct damage to him from the book where his cloning techniques were quoted would have been difficult to prove, according to observers. Only the threat of punitive damages forced the hasty end to the trial.

Michael Stein

at least two reactors built there was also pumped into the lake. Fuel-rod cladding failures were probably frequent, the report says, so the rods leaked material. By 1953, the lake had become dangerously radioactive.

The lake fed into the Techa River, a slow-moving stream along which lived a primitive Bashkir people. Soon, the Techa, too, became "a chronic, waterborne source of radioactive contamination" long before any of the dates of alleged disasters. To separate plutonium from the irradiated fuel elements would have needed nitric acid. The off-gas belched yellow smoke out from a high smokestack, 24 hours a day for

years on end. Because of the high humidity of the region, the Los Alamos theory goes, the nitrogen oxides in the gas-would have produced acid rain and acid-laden snow which could have caused the "dead" landscape seen by Tumerman.

The Soviet Union was in such a hurry to get as much bomb-grade material as possible that the irradiated fuel elements were not allowed to cool, so the gases from the plant would have included radioactive iodine as well. The authors of the report say that the impact of the consequent acid rain would have been appalling. A third source of contamination was the burial of radioactive wastes from the late 1940s and the 1950s. The wastes were not put in steel tanks as at Hanford but radioactive liquid waste was simply dumped into a dry lakebed 5 or 6 kilometres south-east of the plutonium complex.

People in the Techa River region began having radiation sickness, crops were killed by acid rain and the stackgases. So, the report says, the authorities must have evacuated the Bashkirs and burned their homes. This would account for the burned buildings, and for the disappearance of local villages — signs Medvedev interpreted as indications of a nuclear disaster.

The Los Alamos report, like others, confirms that the landscape of the region was changed mightily in the late 1950s. The report includes "before" and "after" maps showing that new lakes were created, drainage canals dug to circumvent other lakes, and so on. All this, it seems, was necessary to stem the contamination of the Techa.

If there were explosions, the report concludes, they would have been chemical, not nuclear. Likely causes cited include ammonium nitrate and hexone, a flammable solvent often used in waste separation.

The report is the latest in a chain of Western "explanations" for the disaster. Curiously, one of the report's two authors, Danny Stillman a Los Alamos staff member, was a co-author of an earlier Los Alamos report attributing the disaster to fallout from "dirty" atomic weapons tests to the north at Novaya Zemlya, and a meteorological fluke that dumped the fallout southwards. Stratton was not available to discuss why the theory has changed: nor was the other author, Diane M. Soran. However, Harold Agnew, a former Los Alamos director who is president of General Atomic Corporation, and who coauthored the Novaya Zemblya theory, says the new theory is an improvement.

The proponents of the buried nuclear waste explosion theory are not convinced. Medvedev says that the report is simply wrong. The people who lived in the area were not simple Bashkir tribesmen as claimed in the report — the Bashkir Republic is somewhere else: the villages that disappeared after the disaster all have Russian names, and therefore had Russian inhabitants.

Moreover, Medvedev says his notion all

along was that the nuclear waste had heated and exploded thermally, or perhaps chemically. Especially during the first two years, nuclear wastes can heat up and possibly explode, unless they are carefully cooled. Medvedev notes that his theory, and a study published by the US Oak Ridge National Laboratory in 1980, conclude there was a single, major disaster on the basis of the ecological literature — evidence that the Los Alamos report ignores.

The Los Alamos report asserts that the incident is relevant "in the light of the current nuclear waste disposal questions facing the United States" but does not explain just how. But it implies that even when nuclear wastes were disposed of in the sloppiest manner imaginable, they caused no single catastrophe. Deborah Shapley

Britain in space

Up, up and away

The British government, not usually enthusiastic about spending public money, seems keen to find the cash for a major national programme in remote sensing. Members of a "task force" who have spent the past six weeks working out the details, were putting the finishing touches to their report earlier this week.

Behind the government's enthusiasm is its wish to make the best use of the European Space Agency's (ESA's) Earth Resources Satellite (ERS1) to which it has already pledged £28 million, or 13 per cent of the cost. Britain does not seem to be the only country to find an interest in remote sensing. All ESA's 13 member states are expected to meet next week's deadline for pledging their contributions to the satellite, even though it will be built under ESA's optional applications programme.

Assuming that the money is forthcoming, ERS1 will be a preoperational ocean-monitoring satellite following along the lines of Seasat, the United States equivalent, which hinted at the usefulness of looking at the oceans in microwave before it went out of service after only three months.

Thus the three instruments that will make up the ERS1 payload — a synthetic aperture radar, a wind scatterometer and a radar altimeter — will all record in the microwave band. The satellite's low polar orbit will give it global coverage for measuring such variables as wind speed, wave height and polar ice cover. Data are expected to be of use to the scientific community, weather forecasters and shipping and offshore oil companies.

Britain's singular interest in ERS1 was stimulated last month when a team from the Science and Engineering Research Council's Rutherford Laboratory won a competition to design a fourth instrument for the payload — an infrared scanning radiometer to measure sea-surface temperature to within half a degree Kelvin.

But the instrument, costing about £1 million, must be built with British money.

If the general level of interest is anything to go by, however, the Rutherford team has little cause for anxiety. The "task force", which includes representatives from the Science and Engineering Research Council, the Natural Environment Research Council, the Meteorological Office, the Royal Aircraft Establishment at Farnborough, industry and the Department of Industry, has been drawing up plans for a far grander national remote sensing programme that could involve spending more than £4 million. The report addresses three questions: the content of a programme of fundamental research into understanding microwave data; how to get the data to the user; and how to exploit commercially remote sensing in general.

The most costly consideration could be that of disseminating data from ERS1. ESA plans to set up a central ground receiving station at Kiruna in Sweden from which data can be relayed to agencies in individual nations. But the British study describes several options for a national ground station that could receive data directly from the satellite. These range from a £4 million station capable of receiving 120 megabits per second from the synthetic aperture radar to a much cheaper station capable of handling data at no more than I megabit per second. The cost would almost certainly have to be met by the industry department, which may try to persuade industry itself to chip in.

The data-handling part of the national programme could ride on the tide of the British government's general enthusiasm for information technology, so that it is no surprise that the report will be placed before Kenneth Baker, minister for information technology. The other important aspect for the government, however, is how to make money out of what is widely heralded as a growing international market. One option that the industry department is considering is to persuade private industry to set up a company to sell remote sensing data, as well as hardware and software for ground stations and satellite payloads.

The final details on the form of the national programme will probably take some months to work out. In the meantime, the plans seem to beg two questions. Are remote sensing data sufficiently attractive for potential users to pay for them? And is Britain, which has no national space agency, sufficiently well organized. On the first point, the industry department seems aware that it will be treading in the dark. But an earlier survey of more than 100 potential users has persuaded it that the risk could be worth taking, especially for ocean monitoring. And the civil servants who have been debating the merits of a national space agency for some time, could be encouraged by a decision to embark on a remote Judy Redfearn sensing programme.

Polish universities

Warsaw's rector

Poland's Council of Ministers has resumed consideration of two bills aimed at restructuring Polish higher education, apparently undismayed that the replacement of Dr Henry Samsonowicz as rector of Warsaw University is regarded in academic circles as a confirmation of growing fears for the future of scholarly life.

Dr Samsonowicz became rector in September 1980, under a compromise arrangement between the old system of government appointment of rectors and the promised reform by which the rector would be elected by the academic community. Subsequently, he was confirmed in office by a free election in which academics, students and university auxiliary staff were represented.

Since the imposition of martial law, Samsonowicz has been under increasing pressure and, according to some sources, was temporarily detained during the round-up of intellectuals in the first few days after the military take-over. A few weeks ago, he was expelled from the Polish United Workers' Party, since when his dismissal has been thought to be only a matter of time.

Nevertheless, the announcement that Dr Samsonowicz's "resignation had been accepted" caused a major wave of protests by Warsaw academics and students. At one point, all lectures were stopped in Warsaw University for 15 minutes to allow students and staff to sign protest letters.

The new rector, selected by the Minister of Science, Higher Education and Technology, is Professor Lazimierz Albin Dobrowolski, head of the department of zoology and ecology. In spite of the unfortunate circumstances of his appointment, Professor Dobrowolski does not seem as severe a hard-liner as some pessimists have assumed.

In a discussion on the proposed reforms of academic life in November 1980, he accepted as necessary the demands for reforms at all levels of education. He even implied that, theoretically, the universities already possess the right to make changes in their syllabuses which, if true, would make many of the reforms promised by the Lodz accords of February 1981 merely a restatement of powers which the universities already possessed but had not used.

With martial law, the reforms were cancelled and university life reverted to the statutes of 1958, which place full administrative power in the hands of the rector and reduce university senates and faculty councils to a merely consultative role. Many features of the pre-Lodz syllabuses, including compulsory Russian, have been restored.

How far the proposed new law will embody these "temporary" measures is not clear. As early as last summer, the then minister, Dr Jerzy Nawrocki, tried to emasculate the promised bill by last minute amendments which he introduced unilaterally. At that time, the minister's action was thwarted by the threat of a nationwide university strike. Strikes, of course, are now illegal under martial law.

Vera Rich

Planetary science

Going downhill

Washington

Ever since Mr George A. Keyworth II. the President's science adviser, made some remarks to the effect that it had already had its day, planetary science has been struggling to find favour in Washington. Its trials are continuing, for its traditional patron, the National Aeronautics and Space Administration (NASA), is cutting back support, and it is not clear that the National Science Foundation (NSF), the logical alternative patron, will take up the slack. Although much more than hardware is involved, the controversy centres at the moment on the imminent closure of the relatively new Infrared Telescope Facility (IRTF) atop an extinct volcano on Mauna Kea, Hawaii.

Clearly, the entire field of astronomy is not going to get everything it wants from the government. For instance, a major study for the National Academy of Sciences (NAS) of future astronomical needs, chaired by George Field, director of the Harvard Smithsonian Center for Astrophysics (see *Nature* 8 April, p.482), has produced a list of desirable future facilities requiring \$1,700 million in expenditure over the next decade. The problem, however, is how to set priorities — a task scientists are reluctant to take on, with the result that it falls to the budget men in Washington.

Earlier this year, the Office of Management and Budget (OMB) told NASA that it could have only two of its three major efforts in astronomy. NASA opted to keep the space telescope, scheduled for launch in 1985, and the science programme for the space shuttle. This means that NASA's third major effort, planetary science, must suffer: the Earth and Planetary Sciences Division of NASA will be cut by half in 1983 compared with 1981, if the proposed \$61 million cut is not restored when Congress considers the NASA budget later this year.

The cuts affect funds that support young graduate students entering planetary science and astronomy, and small grants to astronomers around the United States, away from major facilities. Among the programmes axed was the Venus Orbiting Imaging Radar (VOIR) and funds were not restored for a US Halley's comet probe to complement the Giotto project. Moreover, although support for the solar-polar mission has risen to \$21 million, the increase supports only American work aboard the European spacecraft.

But the most conspicuous cut in the fiscal 1983 budget was the IRTF, which sits 13,000 feet above sea level on Mauna Kea. The IRTF was a pioneering facility built by NASA to observe the planets as support for Voyager and other missions. Its tasks include monitoring volcanism on Io and plotting the infrared signatures of the outer planets and their possible moons.

Axing the IRTF was not an arbitrary decision. All NASA facilities are supposed to support actual missions, and no US spacecraft will encounter a planet until Voyager reaches Uranus on 24 January 1986. Moreover, according to a policy statement made some years ago, all ground-based telescopes funded by the government should be supported by NSF, the government's basic research agency.

On 1 January 1983, the IRTF will be homeless, patronless and mothballed unless Congress restores its \$1.7 million annual operating cost in the NASA budget. NASA has recommended that NSF picks up this tab, but the NSF budget for 1983 is more or less set. Mauna Kea's administrators will have to file a proposal to NSF to get the money — but even then may well not succeed.

John T. Jeffries, director of the Institute for Astronomy at Mauna Kea (which includes other facilities besides the IRTF), is urging Congress to restore the funds to NASA. For the moment, Jeffries says he is reluctant to ask NSF for the money, which at this late stage could come only from other astronomers.

If the telescope goes, United States astronomers would have no national centre for infrared work — either within the Solar System or outside it. The infrared facilities at Wyoming and Arizona serve mostly local astronomers, while a comparable facility on Mauna Kea, the United Kingdom Infrared Telescope, is available mainly to British astronomers.

The Mauna Kea IRTF's plight is typical of the dilemma facing much of the basic science carried out by mission agencies in the United States. The facility is built and operated by NASA, so dedicates half its work in support of NASA's planetary exploration programme. But applications to work on objects outside the Solar System, such as the gas clouds of Orion, are more than double IRTF's applications for planetary work.

But whatever the merits of Mauna Kea's IRTF or the fate of Jeffries' attempts to save it, the issue not being discussed is the one Keyworth raised originally, of whether planetary science has had its day and deserves lower priority than other branches of astronomy. According to Field, chairman of the NAS study, "traditionally, NASA has tried to encourage cooperation rather than conflict between planetary research and galactic and extragalactic astronomy. It has not allowed them to come into conflict but has maintained constituencies for both."

Deborah Shapley

Biotechnology

Dutch go-ahead

Waalre, The Netherlands

The Dutch have at long last got their "innovation programme" biotechnology, eagerly awaited since the beginning of the year (see Nature 14 January, p.91). A government-sponsored committee under the chairmanship of Professor R.A. Schilperoort, who works on recombinant DNA research at the University of Leiden, did not, as might have been expected, recommend setting up new centres for biotechnology along the lines of those planned in microelectronics. Instead his committee has opted for the rapid strengthening of cooperation between government institutes, universities and industry.

The committee urges that the government should provide extra support to stimulate innovation in biotechnology — at least an extra 75 million guilders between now and 1988. This money would come from the government's fund for industrial innovation and be in addition to the planned biotechnology budget, which is much larger.

Efforts should be concentrated on applied research, the committee concludes, especially in sectors where Dutch companies are traditionally strong — such as agriculture, the dairy industry, fermentation and antibiotics production. Areas highlighted for future research include the development of host-vector systems for use in applied research, somatic cell hybridization, second-generation biotechnological reactors for enzyme production and the isolation of useful products from process liquids.

Professor Schilperoort is confident that the existing large and medium-sized Dutch companies (such as Shell and Unilever) will be better able to tackle the challenge than would newly-formed small companies. Discussions on the regulation of recombinant DNA research went on in The Netherlands for far too long. Professor Schilerpoort believes, badly holding up research; "The discussion should stop now. There certainly must be rules, but not stricter than necessary or stricter than in other countries. Permits to start work should be issued quickly, and the rules should be established nationally. Rules made by regional safety authorites have sometimes been too strict.'

Schilperoot, who himself sometimes acts as an adviser to a foreign company, says that Dutch industry and universities often have extensive contacts abroad at the expense of national cooperation. "Better structures here would create a favourable climate that would encourage more confidence in this field and put The Netherlands in a strong position in 5 years' time and have a great impact on society and industrial activity in the next 10 to 20 years."

Casper Schuuring

Shake-up plans for National Academy of Sciences

Washington

This week, the US National Academy of Sciences (NAS) holds its annual meeting in Washington. Probably more than 300 distinguished scientists will gather. They will not transact much business of substance, but there will be much chat about the Academy's reorganization and the new vision of things of its President, Frank Press, a geophysicist who was President Jimmy Carter's Science Adviser.

The NAS is old by American standards (it was founded by President Abraham Lincoln in 1863) and has been accused of being old-fashioned, especially when it tries to give the government the definitive word on such controversial topics as marijunana use or ozone or acid rain. Clearly the academy's science panels have had difficulty giving advice when the assigned topics have been of emotional national concern. "Most of what the academy has done in the past has been extremely good and very important," says Press. "But if we do fifty reports well and one report badly, often the one report gets all the attention.

Press wants to retain NAS's reputation for fairness, impartiality and as the country's most authoritative source of science advice. He wants to continue the special relationship the NAS has long held as the government's science advisory group. But he wants reports to come out faster, review procedures to be simpler, and the NAS to exercise leadership in what topics are studied by having independent sources of income to fund self-initiated studies.

These views have already had their effect. Several key studies were initiated, either with Press's advice before or after his taking office, which are half or less fully supported by the government. Among them are:

 University research and national security headed by Dale R. Corsen, former president of Cornell University. It is due to report in a year;

"DON'T TALK TO ME ABOUT AGEING!"

 Government relations with research universities, led by Burke Marshall, professor of law at Yale University. It will study indirect costs, Circular A-21, federal regulation of the laboratory, and will report this summer;

 Relations between the industrial democracies in an era of high technology competition, led by Howard Johnson, Chairman of the corporation, MIT. It is due to report by the end of 1982;

 Arms control and international security, led by Marvin Goldberger, President of California Institute of Technology;

 Ageing. This study is just getting under way. "The government wasn't interested in supporting this" says Press.

Press has started to raise money for NASA's endowment, most of which was raised by former president Frederick Seitz and which now stands at about \$30 million. Three foundations have given \$3 million so far and a fourth, the Alfred P Sloan Foundation, has agreed instead to help fund the government-research university-study. Meanwhile, NAS's sister organization, the National Academy of Engineering and the Institute of Medicine (IOM), are starting fund-raising drives of their own.

Press is also planning to seek funds from industry through a corporate affiliate programme. "People will say how can you separate donations from control of the work" Press said, anticipating criticism of this move. "We plan to have companies put their membership fees in a blind pool, which we could then draw on without corporate advice to fund our work." In return, member corporations would be invited to special symposia and get all academy reports.

Press, conscious of criticism that academy reports come out slowly and that review procedures are cumbersome, intends to have a special management procedure for the 20 or 30 reports that are nationally significant, thus ensuring that they are "timely" and "dependable".

The studies could be revised faster — "a review should not take more than 2 or 3 months" says Press. Usually, the board overseeing a study does one review of it; then it goes through an separate review procedure run by the National Research Council, NAS's research arm. Press is experimenting with having both reviews go on at once or having the board waive its review if it has a say in the review run by NRC. Another way to speed up reports is not to insist on consensus, but to let dissenters express their views separately. "I've encouraged that", Press says.

To "simplify" NAS procedures, Press is abolishing all the assemblies set up by Handler ten years ago, and two of the future commissions. An *ad hoc* panel under James Ebert, president of the Carnegie Insitution of Washington, looked

at the academy structure and recommended changes, most of which have been implemented by NRC in the past month or so (see *Nature* 1 April p.385).

Under the "streamlined" NAS organisation the old established offices such as the Transportation Research Board and the Office of International Affairs will become their own bosses again, their parent commissions abolished or merged with other things. Agriculture is given new priority, and will probably become its own research board. More attention will be paid to primary and secondary level technical education by the Commission on Behavioural and Social Sciences and Education. What will happen to the



Frank Press - businesslike approach

Institute of Medicine and the Assembly of Life Sciences is uncertain, although there will probably be a new life sciences commission, excluding agriculture. Oceans activities will be consolidated from four boards into two.

Press can implement these changes without a vote of the membership and indeed has already done so. One reason he is seeking non-government money, he says, is to stabilize the staff situation; he plans to hold more regional meetings of members. While most members have accepted the changes, and probably welcome the additional outside funds, Press's business-like approach to the academy has aroused some murmurs — especially from people in commissions being abolished.

Deborah Shapley

Correction

Professor E.A. Barnard, not Professor B. Hartley, is head of the biochemistry department at Imperial College, London (*Nature*, 1 April page 382). Nature apologises to both professors.

CORRESPONDENCE

University role

SIR — Your analysis in Science in France (Nature 25 March, pp.285-304) prompts me to respond, especially on the subject of the role of the universities in the research effort.

First, your articles clearly transmit the French media's impression that the best research in the country is done outside the universities in the grand organismes research centres. Nothing could be further from the truth: many of the most prestigious groups of CNRS and INSERM are directed by university professors (such as P. Chambon, J. Dausset, J.F. Bach, M. Seligmann . . .), and they are also closely involved in the training of future investigators.

Many CNRS and INSERM laboratories are actually located in university campuses, and research teams usually include both university staff and CNRS and INSERM appointees. In addition, many of the CNRS, CEA and INSERM laboratories located outside of the campuses are staffed in part by university faculty. Thus at the Institut Pasteur or at Institut de Recherche en Biologie Moléculaire from the CNRS, a number of teams are directed by university professors, actively involved in the training of the students of the various investigators.

A second point arising from your articles is the future role of the universities in research. The faculties of sciences have been heavily involved in both research and training in the past and will continue to be so. This does not go without a rigorous selection of potential candidates, a fact which remains generally unnoticed by the media: our own "UER de Biochimie" (Unit for Eduction and Research in Biochemistry) has about 700 undergraduate students: only 70 are admitted to the graduate courses in microbiology, virology, immunology, molecular biology and nutrition. The first three of these courses are organized by the University of Paris's staff on the premises of the Institut Pasteur.

Incidently, most of the graduates will end up in CNRS and INSERM research, not only because no new openings are available at the universities but also because these students are the best trained in the field — a fact which we would like our new ministers to be aware of.

A. DONNY STROSBERG

CNRS Institut de Recherche en Biologie Moléculaire, Université Paris VII, Paris, France

Mosquito war games

Sir — In his report (Nature 11 March, p. 104) about allegations of American biological warfare research in India and Pakistan, Dr K.S. Jayaraman is altogether too modest in omitting mention of his own primary role in the press campaign on this topic in India in 1974-75. That campaign was long ago shown to have been based on incorrect information and erroneous reasoning (Nature 252, 342; 256, 355; 258, 102; 273, 96). However, as a result of the atmosphere of suspicion created by that campaign all sorts of difficulties are still put in the way of international collaborative work in India on topics of vital importance to the Indian people, such as nutrition and malaria.

Jayaraman correctly states that yellow fever

does not occur in India and he suggests that the only reason to study the "yellow fever mosquito", Aedes aegypti, would be in support of biological warfare. However, as he was told in 1974, Ae. aegypti is also the vector of dengue and dengue haemorrhagic fever in India and elsewhere. He was always inclined to belittle the importance of these diseases but certainly Literaturnaya Gazeta does not do so, and one of its more bizarre claims was that mosquitoes were being reared in Pakistan and transported to the Caribbean area for use in biological warfare against Cuba, using dengue virus.

It has been claimed that work was planned or carried out at the Pakistan Medical Research Centre (PMRC) on genetic manipulation of Ae. aegypti and on Japanese encephalitis virus. However, Professor R. Baker, ex-director of PMRC, the Annual Reports of PMRC and our own research visits there make it quite clear to us that this was not so. A small amount of work was done on West Nile virus which is indigenous to Pakistan. This work was to study the feasibility of producing a mosquito strain not susceptible to transmission of this virus. Such work may lead to vector control by the introduction of nonsusceptibility genes into wild mosquito populations. The main work of PMRC was on methodology for Anopheles and Culex control by release of semi-sterile males and several elegant genetic sex separation systems were developed to ensure that only males were released. Male mosquitoes do not bite and therefore could not possibly be the agents of biological warfare.

PMRC has produced a remarkable amount of excellent published work. It would be a shame if the history of the WHO/ICMR unit in Delhi should be repeated and the work of PMRC were hampered or terminated as a result of ill-informed and prejudiced journalism.

G. DAVIDSON, C.F. CURTIS G.B. WHITE & P. RAWLINGS

London School of Hygiene and Tropical Medicine, London WCl and Tsetse Research Laboratory University of Bristol, UK

Propaganda wars

Sir - Yet again a leading article your journal (1 April, p.380) disparages the work of Scientists Against Nuclear Arms, the Medical Campaign Against Nuclear Weapons and other groups of professional people dedicated to reducing the present dangerous accumulation of nuclear weapons. The charges brought against these groups seem to be the following: They can only partially address the problem and offer no practical advice; they claim a spurious unity among the profession concerned; they are prone to oversimplifying the issues; and finally, their influence is somehow "irrational". These charges are no more than risks (some more real than others) to be avoided. They are not sufficient reason to condemn the existence of these groups. Perhaps a paragraph's explanation may allay fears that the emergence of these groups is unhelpful or even dangerous.

Education is obviously a place where the informed should act — not individually but in

groups which can bring the information to the public more effectively. This is made even more necessary by the fact that the government's own literature on nuclear questions seems bent on misinformation and deception. As scientists, we have an especial responsibility to inform the public not only because of our knowledge of relevant scientific matters but also because of our understanding of the armaments industry and the complex ways in which technology, warfare and politics interact. Thus, aware of the dangers inherent in the present situation, we need effective representation of our views. This is provided by the professsional groups which are now being formed. Moreover, it is essential to the disarmament movement that it is seen to be as widely based as possible, including professional people as well as parents, Christians, politicians and so on. Diversity here is strength, not weakness.

There are many of us, well informed on the present situation, who are convinced that we shall die within the next few years in a nuclear conflict unless the course of current events can be turned. There are a few signs of hope however, one of which is the emergence, rapid growth and energetic activity of the professional groups that you misguidedly choose to malign. I ask that your journal supports the work of these groups so that mankind can again be confident of its future.

D.E. RYDEHEARD A. MYCROFT

Edinburgh University, Edinburgh, UK

Sir — Your editorial of 1 April ("Professional propaganda", p.380) concludes that groups such as Scientists Against Nuclear Arms, Teachers for Peace and the Medical Campaign Against Nuclear Weapons are deceitful and intrinsically undesirable because they foster the false impression that their professions are united on the issue of nuclear weapons, and because they do not address the relevant issues. This is a contentious inference based on dubious assertions, and it is unusual for Nature to adopt a deliberately provocative and weakly argued position so firmly. This political use of your unique position in scientific journalism seems to me a more egregious abuse of professional influence than the overtly political activities of the scientists and other groups that you have attacked in JULIAN PETO

The Radcliffe Infirmary, Oxford, UK

On the flat

Sir. — M. Hammerton (*Nature* 18 March, p.192) asks why you continued so long to publish letters on creationism, arguing that you would not give such space to flat-Earthers, for instance. But on the same page is a long letter from Professor J. W. Jeffery, citing, as the core of his argument, an unpublished study of his own and a 1979 work of sci-fi and crystal-gazing in energy technology as if it were a work of economic fact.

Surely it is time we heard from the flat-Earthers.

DAVID FISHLOCK

Financial Times, London EC4, UK.

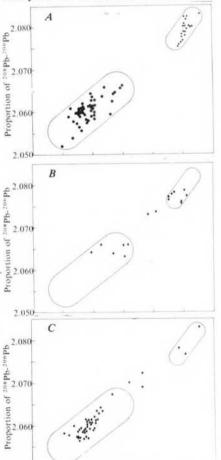
NEWS AND VIEWS

Lead isotopes and the Bronze age metal trade

from Keith Branigan

For the past twenty years archaeological scientists have spent much time and effort trying to trace the metals used by early man back to the ore sources from which they were originally recovered. Attention has been concentrated on the possibilities of identifying characteristic patterning of trace elements in the metal. Research published within the last twelve months by Noël and Zofia Gale of Oxford University, however, has shown that successful provenancing of metals is now possible by using lead isotope analysis. Preliminary results have already produced some major surprises about the supply of metals to the civilisations of the Aegean Bronze Age $(\sim 2000-1200 \text{ BC}).$

Despite the production of many thousands of trace element analyses, both archaeologists and archaeometallurgists have remained sceptical of the successful application of this method to the provenancing of ancient metals. The chemical composition of ores seems to vary within the same ore body, and early smelting techniques resulted in poorly controlled changes in concentrations of trace elements in the metal they produced. An alternative method has therefore been sought by Noël and Zofia Gale, working from the Department of Geology and Mineralogy at Oxford. Their studies have concentrated on lead isotopes which have two important characteristics. First, the lead isotope composition of different lead ores differs according to their geological age and the amounts of uranium and thorium initially present. Thus a lead ore body can be 'finger-printed' by isotope analysis. The second significant characteristic of lead isotopes is that their composition pattern is not altered by the metallurgical processes. This means that not only ores, but also objects made of lead, silver, or other metals containing lead can also be 'finger-printed', and related directly to an ore source. Five years of research enabled the Gales to publish the 'finger-prints' of various Aegean leadsilver sources in June 1981 (Scient. Am. 244, no. 6, 176), and to announce the isotope analyses of about 90 Bronze Age artefacts of lead and silver from the same region. The analyses of ores revealed that lead-silver ores from the islands of Siphnos, Antiparos and Syros, and from the site of Laurion in Attica could all be clearly distinguished by their varying isotope composition patterns (Fig. A). The analyses of artefacts immediately



0.828 Proportion of 207 Pb. 200 Pb.

A, isotope analyses of lead ores from Siphnos (triangles) and Laurion (circles) cluster in two quite distinct groupings (simplified from Gale & Gale 1981). B, lead isotope analyses of 16 lead artefacts from the Cyclades, ∼ 2500 BC, show 8 were made of Siphnian lead, 6 of lead from Laurion and 2 cannot yet be provenanced. C, analyses of 46 lead/silver objects from Crete and Thera (Cyclades) show that by ∼ 1500 BC, almost all objects were made of lead from Laurion on the Greek mainland (field at bottom left).

produced a major surprise - the importance of the Laurion lead-silver source in early Aegean metallurgy. Its significance to classical Athens is well known and documented, but no one had believed that its metals had been extensively used 1.000 years earlier. In fact, the Gales' analyses showed that even in the early Bronze Age 2.000 years before classical Athens the Laurion mines supplied silver and lead to the islands of the Cyclades, the rest of Cycladic silver and lead coming mainly from Siphnos (Fig. B). In the second millennium BC, the Siphnos source was scarcely used and lead and silver throughout the Cyclades and Crete, as well as on the Greek mainland, came mainly from Laurion (Fig. C). But Laurion silver and lead travelled even further afield, for among the artefacts analysed by the Gales were six from Egypt, all of which proved to be made of the distinctive Laurion lead and silver. The earliest of these items dates to the 10th Dynasty, about 2100 BC, and supports other archaeological evidence which points to an opening-up of Aegean trade with the east Mediterranean in the centuries either side of 2000 BC.

At the 5th Creteological Congress in September 1981, at Ayios Nikolaos, Crete, the Gales presented two further papers (not yet published). Zofia Gale described new isotope analyses for lead-silver ores from Crete (characterized in each case by a very high 207 Pb/206 Pb ratio) and also some 40 new analyses of Minoan lead-silver artefacts. They showed that whilst most of the early Bronze Age items in Crete were made of Siphnian lead/silver, a few items of Laurion metal were already reaching the island. Again, this supports the evidence of imported mainland pottery at Knossos around 2500 BC, and of Minoan-style (if not Minoan-exported) trinkets at Ayios Kosmas in Attica at the same period. One major controversy remained unsolved by the analyses - the origin of three silver daggers found at Koumasa in Crete, and variously claimed as Italian, Cycladic and Minoan in manufacture. Zofia Gale's analysis of one of these daggers showed it was made of neither Siphnian nor Laurion metal; its origins cannot yet be determined.

Noël Gale's paper to the Congress and a newly published article in *Science* (216, 11; 1982) are potentially the most significant

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for archaeology world wide, however, for they present the first analyses of copper and bronze artefacts by the lead isotope method. Copper and bronze were used for far more artefacts in antiquity than was lead or silver, and in much of prehistoric Europe, for example, lead and silver were scarcely known. Gale's demonstration that the amounts of lead found as an accidental inclusion in coppers and bronzes is sufficient for isotope analysis opens up wide possibilities of identifying prehistoric metal trades with confidence for the first time. Copper sources used by the Minoan bronzesmiths have been the subject of much debate, and Italy, Crete and Cyprus have all been proposed, with Cyprus the undoubted favourite in the late Bronze Age. Gale has analysed copper ores from Crete, Cyprus and Laurion and established their isotope composition patterns. The first batch of 22 copper/bronze artefacts analysed, all from Crete, surprisingly suggested that there were perhaps four

different ore bodies producing the metal from which these objects were made. although the location of only one of them can yet be fixed — and that is Laurion! Half of the objects analysed come from Laurion, which has never previously been thought of as a potential source of copper for the Aegean civilisations. None of the artefacts was definitely of Cypriot origin, and this result, preliminary as it may be, must raise afresh the old controversy over the famous ox-hide ingots of copper. Found in Cyprus, Crete, Turkey, central Greece, Sicily and Sardinia, and shown in Egyptian tomb paintings, these heavy slabs of copper clearly represented a major Bronze Age trade in metal. But did they go from the west Mediterranean to the east, or from the east to the west, or from the centre to both west and east? Most favoured a Cypriot origin, but Gale's analyses carry at least one clear message - we must think again, and await more analyses, before we can finally solve the problem.

Cooperation and conflict in breeding groups

from N. B. Davies

In over one hundred and fifty species of birds, some individuals forgo breeding and, instead, assist others to feed and care for offspring. Such 'helping at the nest' is as an example of 'altruism' because the helpers increase the reproductive success of the breeders at a cost to themselves. Field studies of marked individuals have shown that, in many cases, the helpers are previous offspring of the breeders and stay at home to help their parents raise more broods. Because the young in the nest are close relatives (often full sibs) a helper does increase its gene contribution to future generations through helping. But why doesn't it adopt the alternative option of leaving home and breeding?

Charnovi has pointed out that most birds have biparental care and two individuals, a disperser plus its mate, could normally rear more young than one alone. By leaving home and pairing up a bird could raise more offspring (coefficient of relatedness, r = 0.5) than it could by staying alone at home and helping to raise extra full sibs (where r is again 0.5). If it stays at home a helper thus forfeits half of its potential brood — the offspring its mate would have reared. Are there, then, ecological conditions which might force young birds to stay at home? Two recent papers by Emlen^{2,3} examine the ecological correlates of helping in birds and provide a model for the evolution of social behaviour in breeding groups.

Helping occurs in a wide variety of environmental conditions. Brown⁴ was the first to point out that individuals are more likely to remain on their parental territory in very stable habitats where populations have expanded to the carrying capacity of the environment so that very few breeding vacancies exist. Good evidence comes from studies of the acorn woodpecker, Melanerpes formicivorus. In coastal California the habitat is extremely saturated and during a three year study⁵ no breeding vacancies arose. On average there were five adults per territory because many young had stayed at home to help their parents. By contrast, in the more seasonal Huachuca mountains of south-eastern Arizona there were many vacant territories and most young dispersed and bred⁶.

Helping at the nest also occurs at the opposite ecological extreme, in very harsh and fluctuating environments, such as the arid areas of Australia and Africa, where rainfall, and hence food supply, is very variable and unpredictable. Emlen² suggests that during harsh periods the costs of leaving to rear young are prohibitive for novice breeders and so many stay at home until conditions improve. Measurements show that young white-fronted bee-eaters, Merops bullockoides, stay at home and help their parents when the insect food supply is low, but in times of plenty they leave and breed themselves.

At first sight it would seem that helping at the nest should be a truly cooperative affair; the breeders gain through help with their brood while the helpers increase their inclusive fitness through raising siblings.

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However, Emlen³ goes on to show that there are often conflicts of interest in these breeding groups and he defines the precise circumstances when these will occur. Sometimes the young will increase their chances of survival by staying on the parental territory but the parents will suffer because of increased competition for resources. In this case the young could decrease the costs of their presence by helping as 'payment' for permission to stay. Breeder-helper conflict will be intensified when r between the helper and the nestlings is low (for example, because one or both of the breeders have been replaced). When there are few outside vacancies helpers may be expected to challenge breeders for breeding rights in the group. There is good evidence for this kind of conflict in pied kingfishers. Cervle rudis, where some individuals offer to feed the breeders' offspring with fish but are often chased away. Here the helpers (who are unrelated to the breeders) may be offering food as a payment for access to the breeding female as a potential mate⁷.

In theory, conflict can also occur in the opposite direction. Emlen suggests that this will be especially prevalent in fluctuating environments where, when conditions for breeding are good, the helpers do better by leaving home. From their parents' point of view, however, they only rear grandchildren (r=0.25) if they leave, whereas if they stay they help the parents to raise more children (r=0.5). Forceable retention of helpers may be impossible but the breeders could, in principle, manipulate circumstances in their favour by disrupting the breeding efforts of their dispersed young so that they came back home to help instead. Trail, Strahl and Brown⁸ report destruction of nests and eggs and the murder of nestlings in groups of Mexican Jays, Aphelocoma ultramarina. In these examples, the murderers were unrelated to their victims but the effect is similar to that envisaged by Emlen. Helpers in the Jay groups may feed the young in several nests at once and so a possible benefit of the infanticide is that it increases the number of helpers available for the killer's own nesting attempt.

Emlen provides an elegant explanation for why there is both conflict and cooperation in breeding groups and the behavioural evidence is in qualitative agreement with the theory. To test the model quantitatively, however, we need to be able to calculate the precise reproductive payoffs to breeder and helper if the helper stayed or if it left. All these calculations to date have relied on measurements of natural variation in the population. For example, if groups with helpers have greater success than groups without, the difference is regarded as the helpers' contribution. In a similar vein, the reproductive success of a novice breeder is regarded as a measure of what a helper would achieve if it left home and bred. The problem of using observations on natural

variation to quantify the parameters in the model is one of confounding variables. Helpers which leave home and breed may be better-quality individuals than those that stay at home. Pairs with helpers may be better-quality breeders than those without; indeed, if the helpers are previous offspring, then their presence may be a consequence of the breeders' high reproductive success in the past rather than a cause of present success.

Experimental manipulations are needed to quantify the helper's contribution and the payoffs it would achieve by leaving home. Field workers are understandably reluctant to carry out large-scale manipulations of their study populations because this interferes with the long-term collection of data on natural success. Brown and his co-workers9 have now reported the first removal experiment of helpers in the grey-crowned babbler, Pomatostomus temporalis. Groups with helpers removed did worse than control groups, showing conclusively that the helpers themselves increase reproductive success.

Further experiments of this kind are needed, particularly as different explanations are likely to emerge in different situations. An intriguing case is that of the dunnock, Prunella modularis. where pairs plus a helper have lower reproductive success than pairs without¹⁰. Before we jump to the conclusion that the 'helpers' must in fact be hindering the pair, a removal experiment is needed. An alternative possibility is that helpers only manage to force themselves on to territories with poor-quality breeders; the breeders may have done even worse without the helper!

Finally, it is interesting to note that cooperative breeding often occurs in groups of unrelated individuals. In the Mexican Jay11, unrelated immigrants help feed other pairs' nestlings and defend the territory in the same way as close relatives. With all the recent emphasis on the role of kin selection in the evolution of social behaviour we may have underestimated the importance of mutualism and reciprocation (see ref. 12). In this issue of Nature (p.740), Packer and Pusey show that there is just as much cooperation in unrelated coalitions of male lions as in related groups. Although a male would increase its inclusive fitness even more by cooperating with a brother, it still pays to cooperate with non-relatives simply because large coalitions father more surviving young per male than small coalitions.

from J.M. Rhodes

IF basaltic magma, produced by partial fusion of the Earth's mantle, were to rise through the mantle and overlying crust, ultimately to be erupted from some volcano with no change in composition, it would be avidly sought and studied by petrologists and geochemists intent on deciphering the composition and mineralogy of the mantle. Basaltic magmas such as these, which have made their way to the Earth's surface without change in composition, are usually referred to as 'primary magmas' since they are the parental compositions from which other, more evolved magmas are produced by a variety of processes as the magma cools. crystallizes and ultimately solidifies. They are regarded as a sort of Rosetta Stone to the Earth's interior, and, indeed, to the interior of any planet, providing suitable samples are available.

However, there is little agreement on whether a primary basalt has ever been sampled, and their nature and composition remain subjects of spirited debate even after almost twenty years. With the advent of the plate-tectonics hypothesis, basalts that have been erupted along the vast 65,000 km interconnecting, spreading midocean ridge systems have figured prominently in this debate, and discussion has mirrored the arguments concerning primary magmas in general.

On the one hand, typical mid-ocean ridge basalts are considered the best candidates for primary magmas because of their vast volumetric abundance, which exceeds the products of more conspicuous forms of volcanism by an order of magnitude, coupled with an apparent world-wide compositional uniformity1-3. Alternatively, and currently favoured, is the argument that mid-ocean ridge basalts, and indeed almost all basalts, are far removed from primary compositions, being instead the end products of an extensive and complex differentiation process from a primary picritic magma; a process that may include crystallization, contamination and mixing with other magmas, as the magma makes its way to the surface through the fractures, conduits and chambers of the volcanic plumbing system⁴⁻⁶. Somewhere between these two extremes is the view that only the most primitive basalts sampled from the ocean floor are primary⁷⁻¹⁰.

All these interpretations have their difficulties. Most mid-ocean ridge basalts show both mineralogical and chemical evidence for crystallization at shallow levels within the crust, an observation clearly at odds with a primary status. Objections to the primary nature of 'primitive' basalts come from melting

experiments, which show that their compositions are at variance with the picritic melt compositions predicted to form at depths of over 30 km in the mantle. The absence of picrites amongst samples recovered from the ocean floor is a major obstacle to their acceptance as primary magmas, and has led to a number of cunning models which attempt to explain why these picritic magmas cannot rise through either the crust or a pre-existing magma chamber!1-13.

These difficulties, and those of identifying primary magmas and understanding the processes that relate them to the commonly erupted mid-ocean ridge basalts, are addressed in a recent comprehensive paper by Bryan and coworkers14 dealing with the compositional variations of basalts erupted along the Mid-Atlantic Ridge adjacent to the Kane Fracture Zone. In contrast to most papers on the subject, Bryan et al. argue for a spectrum of primary magma compositions, rather than a single magma type from which all other ocean-floor basalts are derived. They identify at least two distinct compositions that are parental to most other basalts in the Kane Fracture Zone region through complex interaction of crystallization and magma-mixing processes10,11,15. One of these parental compositions is not unlike the proposed 'primitive' basalt primary magma. Although not abundant, it can be recognized as an end-member component in a number of mixed magma compositions. The other, more widely distributed parental magma does not have a 'primitive' composition, and is, in fact, close to estimates of average mid-ocean ridge basalt compositions¹⁶.

Of these two parental magmas Bryan et al. believe that the 'normal' rather than the 'primitive' variety is the more appropriate candidate for a primary magma, thus contrasting with the recent preference for elusive, if not illusory, primary compositions, and returning to the idea that primary magmas should be abundant and widely distributed, and parental to the less abundant, evolved basalts associated with them¹⁷. They point out that although this suggestion may appear at odds with chemical criteria commonly used to identify primary magmas, it is not contradicted by evidence from melting experiments^{6,18,19}, providing the magmas formed at shallow levels in the mantle. The 'primitive' parental basalts, on the other

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Will the real primary magma please stand up?

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hand, are much less abundant, and do not correspond in composition with experimentally predicted mantle melts produced at any depth. Bryan et al. suggest that these 'primitive' basalts are not primary, but are the differentiates of the elusive primary picritic magmas.

Thus, they envision a wide range of possible primary magma compositions, depending on the depth of formation and segregation from the mantle. The corollary to this is that compositionally similar basalts may have been produced in quite different ways, either by direct melting of the mantle or through differentiation of a more primitive melt en route to the surface. This last point may explain the apparent paradox that while compositional criteria require massive amounts of crystallization to produce evolved basalts from 'primitive' or picritic magmas20, phenocryst assemblages and melting experiments commonly point to a much less extensive process. However, others have explained this same paradox as a consequence of mixing 'primitive' magmas with more evolved ones in crustal magma chambers^{15,21}

In keeping with the controversial nature of the subject, it is unlikely that the scheme presented by Bryan and co-workers for primary basaltic magmas from the Kane Fracture Zone region will win universal acclaim. If correct, it has several important implications. Perhaps the most immediately disquieting, particularly to geochemists, is the realization that the commonly used compositional criteria for identifying potential primary magmas may be of little value. More far-reaching, however, and an aspect not explored by Bryan et al., is the implication that if some of the 'normal', widely distributed oceanfloor basalts are primary and not evolved, then substantial portions of the sub-

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oceanic mantle must be a more fertile source of melts, and richer in iron and incompatible trace elements, than popular mantle models suggest. Whatever one's prejudices, it is clear that the concept of multiple primary magmas will receive more attention in the future. In the paper under discussion the authors argue for primary magmas that are either picritic or widely distributed normal mid-ocean basalts as a consequence of melting at various depths in the mantle. Others appeal to schemes of incremental melting of increasingly refractory mantle in an ascending diapir to produce primary magmas with differing compositions^{8,22,23}. To resolve these increasingly complex concepts, careful and comprehensive major, trace, isotopic, petrographical and experimental melting studies of samples from well documented basaltic terrains will be needed. If these are forthcoming, perhaps the primary magma controversy will have disappeared after the next twenty years.

Interferon redux

from Robert M. Friedman, Lois B. Epstein and Thomas C. Merigan

An international symposium on the chemistry and biology of the interferons*, held a few weeks ago, saw rapid progress reported in four main areas of investigation: structure and function of interferons (IFNs) and their genes, animal and in vitro models for IFN action, IFNs in human diseases, and clinical trials with recombinant and natural IFNs.

Charles Weissman (University of Zurich) reported that 14 different genes for human IFN-a are now known, 3 pseudogenes have been sequenced, and 4 others are known that hybridize but, as yet, have not been sequenced. He has now cloned mouse IFN-a, and suggests that, whereas the genes for IFN-α and IFN-β seem to have diverged some 400 million years ago, those for human and mouse IFN-α diverged about 70 million years ago. D. Goeddel (Genentech Inc.) has cloned four genes for bovine IFN- α and he and his colleagues have also cloned IFN-y in Escherichia coli and monkey cells and achieved its cloning and partial secretion in yeast. The gene has three introns and four exons and is located on human chromosome 12.

Natural IFN-y has been purified to homogeneity (J. Vilcek, New York University School of Medicine) and is found in 20 and 25,000 molecular weight forms. 125 I-labelled IFN-y binding studies show the receptor for IFN-y and IFN-β to be the same, but distinct from that for IFN- α . In preliminary antiproliferative studies on HeLa eells, pure IFN-y was no more potent than IFN-α. High-level production of cloned IFN-\(\beta\) (M. Innis, Cetus, Berkeley) is associated with distinct morphological changes in the producing bacteria.

Recombinant IFN-α has crystallized by Weissman's group and by S. Pestka (Roche Institute) and his colleagues, but X-ray diffraction studies have yet to be used to elucidate the tertiary structure of the molecule. From the location of hydrophobic and hydrophilic

*The symposium on "Chemistry and Biology of Interferons: Relationship to Therapeutics" was held on 742 March 1982 at Squaw Valley, California. It was one of the 11th Annual UCL Symposiums on Molecular & Cellular Biology to be published by Academic Press, New York

residues, R. Wetzel (Genentech Inc.) predicted that IFN-a is a globular protein with a hydrophobic core, more than 65 per cent of which is in α -helical formation connected with intervening \(\beta\)-sheets. Both he and Pestka indicated that the last 11 amino acids of the recombinant A IFN are not necessary for its antiviral effect; in fact, Pestka and his colleagues have found three natural IFN-a molecules that are only 155 amino acids long, compared with most other IFN-a molecules which have 165 or 166 residues. Pestka argued that the potency of various IFNs should be defined in terms of the number of molecules necessary for achieving a given effect now that the availability of pure recombinant IFNs makes this possible. His and others' data make clear that various recombinant IFNs have different degrees of antiviral and antiproliferative activity when compared on a per molecule basis. Despite the differences, L. Epstein (University of California, San Francisco) showed that natural IFN-a and recombinant IFN-a (A) induce the same peptides in normal fibroblasts. Recombinant, like natural, IFN- α also induces 1.5-2 times as much of these peptides in fibroblasts trisomic for chromosome 21.

Several groups have inserted the genes for human IFN-β in murine cells and J. Collins and his colleagues (Gessellschaft fur Biotechnology) have found that at least five other genes are co-regulated with the IFN- β gene, but activation of these genes is not necessary for production of IFN-B in this heterologous background.

The antiviral and cell growth inhibitory effects of IFNs can be separated in a Swiss mouse cell clone that remains sensitive to the former, but has lost sensitivity to the latter (T. Sreevalsan, Georgetown University). IFN treatment was also shown to result in a decrease in ADP

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ribosylation and in protein methylation. There was much discussion of the role of the IFN-induced protein kinase and 2',5'-oligoadenylate (2',5'-A,)-related systems in the actions of IFNs. The 2',5'-A_-activated endonuclease is important in determining the antiviral action of IFNs (I. Kerr, Imperial Cancer Research Fund). The endonuclease may also be important in IFN inhibition of cell growth: endonuclease activity was absent in human melanoma cells resistant to growth regulation by IFN, but present in melanoma lines sensitive to it (A. Creasey and T. Merigan, Stanford School of Medicine). The 2',5'-A, system may have other roles: the degree of natural killer cell activity in large granular lymphocytes was increased by transfection with 2',5'-A, (R. Herberman, NIH). P. Torrence (NIH) explored the significance of the IFN-induced kinase in inhibiting in vitro viral protein synthesis. Adding the double-stranded RNA, poly(A) poly(2'-fluoro, 2'-deoxyuridylic acid), that activates the protein kinase, but not the 2', 5'n-synthetase, to extracts of L cells does not cause inhibition of protein synthesis; although the same inducer inhibits protein synthesis in reticulocyte lysates. Several other model systems for IFN activity were presented. In mouse cells transformed by bovine papillomatosis virus, in which the viral DNA remained as an episome, the viral DNA copy number per cell was decreased by IFN treatment (P. Howley, NCI, and R. Friedman). After 10 passages morphological cell revertants, entirely lacking in viral DNA, were found. Such 'cured' cells could be again transformed by the virus.

IFNs were reported to be present in a variety of human diseases, but their origin and effects remain cryptic. An unusual species of acid-labile IFN-a is present in about 50 per cent of patients with systemic lupus erythematosis (SLE) and in male homosexual patients with Kaposi's sarcoma or lymphadenopathy (O. Preble, J.A. Sonnabend (NIH), R. Friedman & J. Vilcek). One SLE patient was found to carry antibody to human IFN-a. The glomerular basement membranes from the kidney of another SLE patient with membranous glomerulonephritis developed fluorescence when sequentially exposed to rabbit anti-human IFN-a and to fluorescent anti-rabbit IgG (S. Panem, University of Chicago). The latter suggested that immune complexes containing human IFN-a may contribute to the development of renal disease in SLE. Another morphological relationship between SLE and IFN was reported by S. Rich who found that tubuloreticular structures, that are induced by IFN treatment of human lymphoblastoid cells, are present in both SLE patients and human cancer patients undergoing IFN treatment. J. Hooks (NIH) described the in vitro production of IFN-y by the tumour cells of a patient with an OKT8-positive

Olivine transformed from J. Zussman

J.D. BERNAL (like W.L. Bragg) applied his knowledge of crystal structures to a broad range of topics and, as early as 1936, was able to suggest that the mineral olivine, (Mg,Fe),SiO₄, would transform to the spinel structure at the high pressures found deep in the Earth's mantle. There is now much evidence that olivine is the major constituent of the rocks of the Earth's upper mantle, so any transformation that occurs at greater depth is of obvious geological and geophysical importance.

In 1966, Ringwood and Major¹ produced the transformation in the laboratory (at about 1.000°C and 115 kbar) and found not one, but two, highpressure polymorphs, β- and y-Mg₂SiO₄. The higher-pressure y-phase does, as suggested, have the spinel structure and the β -phase a modified version of it. Analogous structures for Co,SiO4 were later determined by Morimoto et al.2. Density increases of about 10 per cent are associated with the transformations and probably explain the discontinuity in seismic P-wave velocity3 observed at a depth of about 400 km. The phase change with increased density may perhaps also be a driving force for the downward movement of slabs of oceanic crust at continental plate margins, and may act as a trigger for deep-focus earthquakes in these regions4.

The β - and y-phases have also been found in certain meteorites and are thought to have been produced by extraterrestrial - rather than Earth impact shock pressures. Studies of the Tenham chondritic meteorite by Putnis and Price suggested that the β -phase was metastable, but phase relationships in the system Mg2SiO4-Fe2SiO4 at high pressures and temperatures are particularly complex at the Mg-rich end.

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T-cell leukaemia, and C. Chany has (INSERM) found a variety of IFNs in human amniotic fluids. In addition, L. Epstein discussed several defects in IFN-a or -y production, but saw no reason for treatment of the immunological disturbances in these conditions with IFNs at present, or until there is further understanding of the role of IFNs in the pathogenesis of primary and secondary immunodeficiency diseases.

One purified recombinant DNAproduced IFN, a2, (Hoffman La Roche) stimulated low levels of antibodies in 10 out of 200 treated patients and in this respect, it resembles native IFNs. The National Cancer Institute's Phase 1 trials of recombinant IFN-a2 and lymphoblastoid IFN also indicate some antitumour activity in various human cancers. In contrast to other published results, an increase in natural killer cell activity could not be

It seems likely that the direct $\alpha \rightarrow \beta$ transformation, and also $y \rightarrow \beta$ and $y \rightarrow \beta \rightarrow y$ can all occur with increasing depth because the associated temperature increase enlarges the stability field of the β-phase6

In this issue of Nature (p.729), Price, Putnis and Smith now describe transmission electron microscopic studies of veinlets in the Peace River meteorite. The B-phase occurs in micron-size crystals as pseudomorphs of olivine and also replace ringwoodite (naturally occurring a-Mg,SiO₄). They identify defects in the ringwoodite as stacking faults which are, in effect, very thin sheets of the B-structure in the fault plane. They point out that the β - and γ -structures can, as a whole, be considered related by such stacking faults, and suggest that growth of the β -phase from the fault plane produces fault-free β -crystals. In the meteorite the \(\beta\)-phase is thus derived from the y-phase in a post-shock retrograde process occurring on reduction of pressure. This contrasts with the views of Madon and Poire7 that the faults in ringwoodite are associated with the prograde olivine to spinel transformation.

The authors round off with some speculation as to the relevance of the mechanism of transformation that they describe to a $\gamma \rightarrow \beta$ transformation in the Earth's mantle. They suggest that the systems of stacking faults involved might well be sensitive to external shear forces and thus pertinent to the rheological properties of the mantle.

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demonstrated. Positive results in cancer patients also come from a French trial of IFN-β in which dosage level and scheduling were the minimum necessary to elevate natural killer activity in vivo. Preliminary clinical trials of IFN-B in Germany and Japan were presented, as well as studies of lymphoblastoid IFN produced by the Burroughs-Wellcome group. Gutterman (MD Anderson Hospital) reported a series of 19 renal cell carcinoma cases in which 7 patients showed shrinkage of disease with IFN treatment.

Two newly analysed randomized, controlled trials showed positive results. A group from Stanford observed that varicella virus infections in leukaemic children can be improved with IFN and M. Hirsch (Massachusetts General Hospital) reported that the impact of CMV infection on renal transplant recipients can be lessened with IFN-a prophylaxis.

Breaking the active galaxy speed record

from Andrew Lawrence

OBSERVATIONS of the variability of the light output of quasars and Seyfert galaxies have been of great importance as probes of the physical conditions in these 'active galactic nuclei'. New evidence has been reported! that the Seyfert galaxy NGC 6814 varies on the amazingly short time scale of 100 s, not only revealing the smallest dimensions so far for an active galactic nucleus, but at last beginning to place constraints on the physical processes responsible for the prodigious rate of energy output.

The time during which a body can change its behaviour by a significant amount is limited by the speed with which information can travel across the body; large bodies cannot alter as rapidly as small ones. The early realization that some quasars vary on a time scale of months² led astronomers to the astonishing conclusion that bodies that could fit comfortably between here and the nearest star are

emitting as much energy as an entire normal galaxy. The 1970s saw the discovery that quasars and Seyfert galaxies expend a large fraction of their energy budgets as X rays. Several experiments found cases in which X-ray production varied over days and even hours3-8. Such considerations have led to the common belief that X rays relate most directly to the actual 'central powerhouse' of active galaxies. The volume of space involved in such a fast variation is far smaller than can be directly resolved with either optical telescopes or even the largest baseline radio interferometer. Variability studies may thus extract information unobtainable by any other method

In 1978, controversy began over evidence presented by a group at Harvard⁹

Andrew Lawrence is at the Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex BN27 1RP. that the X-ray emission from the Seyfert galaxy NGC 4151 had varied on the remarkable time scale of 700 s. The data were rather marginal, and a group at the Goddard Space Flight Center (GSFC) published observations repudiating this suggestion⁵. However, a new study by Tennant *et al.*, from the same group at GSFC¹, has presented convincing evidence that a similar Seyfert galaxy, NGC 6814, varies its output on a time scale of 100 s.

Such very rapid variability tells us more than just the size of the radiating body. It places constraints on the parameters of models suggested to explain the activity of these galactic nuclei. Tennant *et al.* discuss several points in detail.

One hundred seconds corresponds to a light travel distance of 3×10^{12} cm. The X-ray emitter cannot be larger than this. If a massive black hole is at the heart of the business, it cannot be more massive than 10^7 solar masses (M_{\odot}); or the event horizon would be outside 3×10^{12} cm. On the other hand, to explain the observed luminosity by accretion of matter requires a black hole of at least $10^5 M_{\odot}$ Such a system can have been radiating at its present luminosity for



100 years ago

ILLUSTRATIONS OF NEW OR RARE ANIMALS IN THE ZOOLOGICAL SOCIETY'S LIVING COLLECTION

HARDWICKE'S CIVET-CAT (Hemigalea Hardwickii). — The Viverridae, or Civet-cats, form a well-marked family of carnivorous mammals peculiar to the tropics of the Old World, and mostly confined to Southern Asia and Africa, though one or two of them occur in the southern parts of Europe. One of the finest and largest of them is the True Civet-cat (Viverra civetta), from the anal glands of which the old-fashioned perfume known as civet is extracted, and the Genets, Ichneumons, and Mungooses are well-known members of the same family, examples of which are always to be seen in the Zoological Society's Collection.

Amongst the rarer and less familiar forms of the Viverrine groups is the very curiouslymarked animal which we now figure (Fig. 17) from a specimen received by the Society in October, last year. Hardwicke's Civet, though first described by Dr. Gray so long ago as 1830, is a very scarce and little-known species, and the present example is believed to be the first of its kind ever brought alive to this country. In 1840 Müller and Schlegel gave an excellent figure and description of this animal, under the name of Viverra boiei, in their great work upon the Natural History of the colonial possessions of the Netherlands. Their specimen was obtained in South-Eastern Borneo by Herr Henrici, and sent alive to the



Fig. 17. Hardwicke's Civet-cat.

Gardens of the Zoological Society of Amsterdam. Hence, after its death, it was transferred to the National Museum of Leyden. Müller adds that he never met with this Civet-cat himself during his extensive travels in the Eastern Archipelago, and had received no information as to its habits.

Hardwicke's Civet-cat was also figured and described by Eydoux and Souleyet in the "Zoology" of the voyage of the Bonite in 1841, under the name Hemigate zebra, but again without any information as to its habits,

not even the locality of their specimen being stated.

So far as has been ascertained from the Zoological Society's living specimen, this animal is excessively shy and retiring in disposition, and apparently does not leave its retreat voluntarily except at night. When handled, it ejects a highly acrid and skunk-like secretion from its anal glands. The length of the body in the example figured is about 24 inches, and that of the tail about 18 inches. From Nature 25, 608, 27 April 1882.

only 2 per cent of the currently accepted age of the Universe or it would have accreted more than its own mass. As approximately 2 per cent of all galaxies are active, the new evidence could suggest that all galaxies have an active phase for 2 per cent of their life times.

Much argument about X rays from active galaxies centres on the radiation mechanism involved. Is it thermal? Or is it due to very high-energy relativistic electrons (like those found in radio galaxies)? Such high-energy electrons must lose their energy extremely fast. Tennant et al. conclude that for such non-thermal mechanisms to be viable, some process must continuously re-accelerate electrons throughout the emitting volume. Are thermal processes simpler? Not really; the X-ray spectrum is consistent with optically thin thermal emission, but from such a small volume, thermal radiation should be optically thick. Thermal processes are only viable in more sophisticated models where very hot electrons give a 'boost' to UV photons inserted from outside the X-rayemitting region.

Why have such rapid fireworks not been seen from other objects? In another, as yet unpublished, investigation10, Tennant et al, find that of 30 objects studied, only NGC 6814 showed such behaviour. In other studies of Seyfert galaxies, only NGC 4051 and possibly NGC 3227 have shown variability on a time scale of minutes^{11,12}. All three galaxies are rather low-luminosity examples of the Seyfert phenomenon, but other low-luminosity objects have not shown very rapid variations. There are indications that these objects have anomalous optical and IR properties13, but the data are still scant and confusing.

Recently preprinted observations with the Einstein Observatory14 have claimed 100 s variability in the quasar 1525 + 227.The data are not as convincing as in other cases, but if confirmed, the new observations could be of great importance; the X-ray luminosity of 1525 + 227 is several hundred times that of NGC 6814, and the constraint placed on models would be correspondingly tighter. Hope for further progress in these matters in the near future rests on the performance of the European X-ray satellite EXOSAT, due to be launched in autumn 1982.

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Receptors for T-cell growth

from D.E. Kipp and B.A. Askonas

THE study of lymphocyte mediators has recently become an active area of cellular immunology. Although it has been known for more than twenty years that conditioned media from lectin-stimulated spleen cells contain lymphokines which enhance in vivo immune responses^{1,2}, the more recent discovery that conditioned media can support the continuous growth of activated T cells in vitro3 has made the T-cell growth factor(s) (TCGF) of particular significance.

In vivo, the importance of TCGF lies in the control of T-cell clonal expansion and responses. In vitro, the use of TCGF has proved of enormous value since it has made it possible to select and grow T-cell clones with different functions, such as cytotoxic T cells or T-helper cells. For the first time, single clones of T-cell subpopulations of known antigen specificity can be studied. This has made it possible to characterize their phenotype and functions, and their release of various lymphokines, factors, or interferon; to type lymphocyte-defined human histocompatibility antigens; and to determine the fine specificity of individual T cells and their recognition of antigen in conjunction with products of the major histocompatibility gene complex. It has also made it possible to provide important sources of lymphokines.

The resting T cell is unresponsive to TCGF and presumably does not express receptors for TCGF on its cell surface. In contrast, mitogen- or antigen-activated T-cells respond to TCGF4 and continue to grow in vitro. In a recent report, R.J. Robb, A. Munck and K.A. Smith⁵ define receptors for TCGF by examining the binding of biosynthetically labelled TCGF derived from a human T-leukaemia cell line (JURKAT) to various cell types. TCGF was purified after incubation of JURKAT cells with radiolabelled amino acids to a single band on SDSpolyacrylamide gel electrophoresis at a molecular weight of about 15,000, equivalent to the previously estimated molecular size of human TCGF6. This was the only measure of purity, but the material was functionally active and its binding properties to cells paralleled functional activity. The authors estimated by equilibrium binding assays of the purified radiolabelled TCGF that there were 10-15,000 receptors on TCGF-dependent mouse cytotoxic or helper T-cell clones. Furthermore, while normal murine splenocytes and thymocytes as well as human peripheral blood cells showed just barely detectable binding of radiolabelled TCGF, alloantigen- or lectin-stimulated cells had

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easily detectable receptors. This result supports the observations that only antigen-or mitogen-activated T cells are responsive to TCGF, whereas unstimulated T cells (and lipopolysaccharideactivated B cells) are not reactive to TCGF and appear not to possess any receptors for TCGF

Robb et al. also studied the binding of radiolabelled TCGF to a variety of human neoplastic cell lines. All of the non-T-cell lines and 90 per cent of human T-cell lines failed to bind TCGF. The human T-cell lines have been classed as T-cell lines on the basis of rosette formation with sheep red blood cells and this may not be an ideal criterion. The absence of TCGF receptors may therefore be explained in several ways. The transformed cell lines may have lost expression of their receptors or may have been derived from early T cells that had not yet expressed receptors. Certainly, antigendependent untransformed T cells, be they helper or cytotoxic cells, can respond to TCGF7.8. The one human T-cell line found to bind TCGF, which apparently grows well without the addition of exogenous TCGF, has recently been shown to both produce and respond to TCGF9.

The work of Robb et al. also confirmed the specificity of the human receptor for TCGF. The binding of radiolabelled human TCGF to its receptor was not inhibited by any other growth factor tested or by TCGF derived from rodent spleen cells which does not act on human T cells. On the other hand-human TCGF is not species specific and acts on rodent T cells as well¹⁰. The association and dissociation of human TCGF binding to mouse cytotoxic T cells showed a rapid turnover of TCGF and its receptor as well as a rapid degradation of TCGF after association with its receptor. This rapid degradation may have an important regulatory significance as the presence of TCGF would allow for the continued expansion of activated T cells in the lymphoid tissues, and thus block any dampening of the immune response when it is no longer needed.

These studies provide insight into the regulation of the immune response by TCGF. However, it needs to be stressed that this is only the beginning of such studies. Clearly it will be important to define the nature of the receptor for TCGF and we thus await eagerly the next chapter of this unfolding story.

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Shear stress in fault zones

from Barry Kean Atkinson

SINCE estimates of the shear stress acting on fault zones at major plate boundaries varv by at least an order of magnitude, such fundamental issues as the driving mechanism of plate tectonics and the energetics of crustal faulting remain entirely unresolved1. The shear stresses in the Earth's crust cannot be measured directly, except very close to the surface, so likely stress levels have to be inferred from various secondary lines of evidence.

Laboratory studies of rock friction generally predict that shear stresses on earthquake faults must be at least as high as 100 MPa, unless pore fluid pressures approach that due to the overburden. Such high pore fluid pressures may occur transiently during earthquake faulting through a shear-heating-induced thermal expansion of water² but an impressive array of data (summarized in ref. 3) shows that pore pressures much greater than hydrostatic will generally only develop in crustal rock sequences containing thick blankets of impervious rocks.

A different level of shear stress is indicated by seismological studies. The average stress drop in crustal earthquakes is usually of the order of 10 MPa, or less, irrespective of the strength of the earthquake source, implying that ambient stress levels are also of this order. The key evidence of long-term, low shear stress, probably around 10 MPa, comes from the absence of a heat flow anomaly around the Andreas Fault. A recent San comprehensive review4 of the latest heatflow data for the western US further supports this view. However, unless relative plate velocities are unreasonably high (tens of cm yr⁻¹), the metamorphic gradients and K-Ar ages of rocks along the Alpine Fault of New Zealand are consistent with shear-heating due to a much higher shear stress⁵, of at least 100 MPa.

Geological evidence from fault rock textures suggests that both high shear stress (100 MPa) and low shear stress (10 MPa) faulting occur in the upper crust, with the latter more common6. High pore fluid pressures are clearly involved in some low stress crustal faulting and higher shear stresses are usually associated with reverse faulting on dry immature fault zones (the Alpine Fault may be exceptionally dry⁷).

Even if earthquakes along plate boundaries often occur in an environment where ambient shear stress is low, it could be argued that intraplate earthquakes are different and occur where the ambient shear stress is higher. In the eastern US, for

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example, some surficial rocks have higher values of deviatoric stress than are found in the western US, perhaps implying shear stresses of several tens of MPa at depth. In addition, the moment of earthquake faulting for a given length of fault break is about one order of magnitude higher in some intraplate regions of the US than in western California. The argument has, however, recently been demolished by Raleigh and Evernden⁸ — for the US at least. They point to some little appreciated facts.

First, attenuation rates of horizontally propagating seismic waves with frequencies relevant to intensity observations (1-4 Hz) are grossly different throughout the US. Second, the energy of intensity-relevant frequencies for earthquakes in the conterminous US is solely dependent on fault length and has no relation to attenuation. Finally, for a fixed length of fault break there is a very substantial increase in seismic moment for a change in attenuation from that typical of western California to that typical of eastern US. Thus, moment values have to be explained within models of the Earth that are highly heterogeneous.

These facts can be explained if earthquakes in eastern US occur along fault zones that constitute soft inclusions in an otherwise rigid and strong crust/mantle system. In order to calculate the true moment of an earthquake, allowance must be made for the effective dimensions of a volume in which stress relaxation occurs. Relatively high moments for short fault breaks are only achieved in association with a large volume of relaxation which can be larger than the inclusion. There is no physical anomaly implied by this result, merely a more heterogeneous earth model than is usually used in seismology.

Raleigh and Evernden⁸ also review the evidence for low shear stress on Californian fault zones and conclude that fault zones in all regions of the US are sites of low ambient shear stress and low stress drop.

Fault motion at low shear stresses has been supposed difficult because high pore fluid pressures have been thought to be the only way to reduce the frictional resistance of a fault. Recent research has shown, however, that the chemical effects of pore water may also have a significant effect.

It is notoriously difficult to run deformation experiments in the laboratory at strain rates comparable with those in tectonically loaded fault zones. Yet, such experiments are essential if the range of potential water-weakening reactions are to be assessed. A few years ago, it was shown that stress relaxation experiments9 could be used to assess strain rates on fault zones down to about 10-10s-1, within one or two

orders of magnitude of some tectonic strain rates but some five orders of magnitude slower than typical laboratory strain rates. Since then this technique has been applied to the study of stress levels on water-weakened faults in a range of crustal rocks10.

For faults in many wet crustal rocks a dramatic weakening occurs at strain rates below 10⁻⁷s⁻¹, probably due to some combination of stress corrosion and diffusional mass transfer. The effect is in addition to any strength reduction due merely to the mechanical effect of high pore fluid pressures. Additional support is given to the stress corrosion hypothesis by the observation that in quartz, the rate of crack growth increases by five orders of magnitude on raising the temperature from 20 to 200°C (ref.11).

Some of these experimental results can be extrapolated to conditions thought to occur at depths down to 15 km along the San Andreas Fault Zone, For sandstone and quartzite, shear stress for sliding under slow, tectonic strain rates of 10-11 to 10-14 s⁻¹ is of the order of 10 MPa, even if pore water pressure never exceeds hydrostatic. For granite, however, a modest pore water overpressure is required to lower shear stress for sliding into the 10 MPa range.

The explanation for diversity of shear stresses on crustal fault zones may lie in water's different mechanical, thermodynamic and, especially, chemical properties. There is sufficient variation both in the time scale of fault zone processes and in the material properties of crustal rocks which, given the diverse physico-chemical actions of water, could explain the range of shear stress estimates for fault zones. There is a strong case8 that the environment in which many earthquakes occur is of low shear stress, but with stronger asperities of varying sizes and density of distribution along the fault surface which account for less common, local shear stress of several tens of MPa.

Future work must concentrate on working out the details of potential chemical weakening effects of pore water on rock strength under simulated crustal conditions. Some reliable means will also be required for extrapolating laboratory experiments to the larger strains typical of slip on earthquake fault zones.

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REVIEW ARTICLE

Atomic and gravitational clocks

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Atomic and gravitational clocks are governed by the laws of electrodynamics and gravity respectively. While the strong equivalence principle (SEP) assumes that the two clocks have been synchronous at all times, recent planetary data seem to suggest a possible violation of the SEP. Our past analysis of the implications of an SEP violation on different physical phenomena revealed no disagreement. However, these studies assumed that the two different clocks can be consistently constructed within the framework. The concept of scale invariance, and the physical meaning of different systems of units, are now reviewed and the construction of two clocks that do not remain synchronous—whose rates are related by a non-constant function β_a —is demonstrated. The cosmological character of β_a is also discussed.

OF the forces of nature, the two more successfully described are the electromagnetic forces, through quantum electrodynamics (QED) and macroscopic gravity, through Einstein's theory of general relativity (GR). The high level of agreement between predictions and observations leave little doubt that we now possess the correct physical interpretation as well as the theoretical tools to describe both forces.

QED and GR are also complete theories in the sense that they yield operationally well-defined clocks which satisfy the dynamical equations of the theories themselves. To understand what is required for a theory to yield a well defined clock, we introduce the notion of scale invariance, SI.

Consider a dynamical equation defined in a given system of units, containing field variables and dimensional parameters. Consider now a scale (length) transformation of the type

$$L \to L_{\pm} = \Omega_{\pm}^{-1}(x)L \tag{1}$$

where $\Omega_*(x)$ is a dimensionless arbitrary function of space-time. Regarding coordinates as dimensionless space-time markers, it follows from equation (1) that

$$g_{\mu\nu}^* = g_{\mu\nu} \Omega_{\star}^{-2}(x), ds_{\star} = \Omega_{\star}^{-1} ds$$
 (2)

Furthermore, a physical tensor Λ of arbitrary rank will be taken to transform like

$$\Lambda \to \Lambda^* = \Lambda \Omega_{\pm}^{-\pi(\Lambda)} \tag{3}$$

where $\pi(\Lambda)$ is called the power of Λ : $\pi(ds) = 1$, $\pi(g_{\mu\nu}) = 2$. Because null cones transform into null cones, it follows that c = 1 holds in all systems of units. Therefore, because v = (v/c)c, $\pi(v) = 0$. The power of any quantity can therefore be expressed in terms of $\pi(m)$ and $\pi(L) = 1$.

Given these definitions, an equation is scale invariant if: (1) in the transformed system of units, it preserves the same form involving the transformed fields; (2) it has the same parameters; and (3) there is no explicit dependence on $\Omega_*(x)$

We now show that a SI theory cannot yield a unit of time, that is, a clock. Because of the assumed SI, the dynamical equations governing the clock are identical in any system of units and so their solution for the period p of the clock in one given system of units is the same in any other system of units, $p = p_*$. On the other hand, if instead of solving the dynamical equations, we apply the transformation (1) directly to p, we obtain the result $p = \Omega_* p_*$, that is $p \neq p_*$, in contradiction with the previous result. It follows that a SI theory yields a solution

that is simultaneously constant and variable, thus proving that such a quantity is physically meaningless. In other words, a SI theory, being invariant with respect to changes in scale, does not possess a scale, thus lacking an intrinsic unit of time.

Therefore, a SI theory cannot provide a clock. For a clock to exist, the underlying theory must be non SI.

As an example, let us consider the case of the electromagnetic clock, an electron revolving around a proton, governed by the following equations¹:

$$\frac{1}{\sqrt{g}}\left[\sqrt{g}F^{\mu\nu}\right]_{,\nu} = J^{\mu}, \qquad u^{\mu};_{\nu}u^{\nu} = \frac{e}{m}u^{\lambda}F^{\mu}_{\lambda} \qquad (4)$$

The periodic solution with the period p is given by

$$p = 2\pi \frac{m^2 l^3}{e^4} [1 + 0(v/c)^2]$$
 (5)

where l is the angular momentum per unit mass. In equations (4) there is a 'time coordinate' x^0 whose physical meaning is not given by equations (4), where x^0 is in fact a marker. Its physical meaning can be determined by considering that since $de/dx^0 = dm/dx^0 = dl/dx^0 = 0$, we have from equation (5) $dp/dx^0 = 0$, thus allowing one to attribute a physical meaning to x^0 . Equations (4) are therefore constructed so as to yield a physical unit of time, given by equation (5). This property is due to the fact that equations (4) are not SI. In fact, let us apply equation (1) to equations (4). As $2\pi(e) = \pi(h) = 1 + \pi(m) = 2 - \pi(G) = 2 - g$ and $2\pi(F_\alpha^\beta) = 2\pi(E, H) = \pi(\rho_\gamma) = \pi(m) - 3$, (E and H are the electric and magnetic field strengths, ρ_γ is the energy density, e^2/hc is a pure number and both h/mc and GM/c^2 have the dimensions of a length), equations (4) become treating coordinates as dimensionless,

$$\frac{1}{\sqrt{g_*}} \left[\sqrt{g_*} F_*^{\mu\nu} \, \Omega_*^{4+\pi} \right]_{,\nu} = J_*^{\mu} \, \Omega_*^{4+\pi} \tag{6a}$$

$$u_{,\nu}^{*\mu} u^{*\nu} + \Delta_{*}^{\mu\nu} \frac{\Omega_{,\nu}^{*}}{\Omega_{*}} = \frac{e}{m} u^{*\lambda} F_{*\lambda}^{\mu} \Omega_{*}^{\pi+3}$$
 (6b)

with $\Delta_{\mu\nu} \equiv u_{\mu}u_{\nu} - g_{\mu\nu}$ and $2\pi \equiv 2\pi(F^{\mu\nu}) = \pi(m) - 7$.

Clearly, while equation (6a) can be made SI by assuming $\pi(m) = -1$, equation (6b) cannot, for the Ω_* dependence in the Δ term cannot be made to disappear because there are no free parameters in $\Delta_{\mu\nu}$. Therefore the system (4) is not SI.

Let us now consider a gravitational clock (a planet and a star, for example) and let us consider the Einstein equations describing macroscopic gravity. From the previous analysis it follows that the lack of SI of equations (4) is due solely to the equations of motion which in the gravitational case are already contained in the Einstein field equations, which can therefore be expected to be not SI. This is indeed the case²: Einstein equations do not retain their original form under the scale transformations (1)–(2). Their lack of SI is why a gravitational clock is a well-defined quantity, whose period, given by Kelper's third law, reads (M is the total mass and J is the orbital angular momentum per unit mass)

$$P = \frac{2\pi J^3}{(GM)^2} \tag{7}$$

The above discussion shows that the electromagnetic and gravitational clocks are meaningful because the dynamical equations governing them are not SI.

The strong equivalence principle

To study the relationship of the two clocks, consider a physical phenomenon characterized by a proper length interval Δs , which we shall measure using electromagnetic and gravitational clocks, the results being Δs_a (atomic) and Δs_E (Einstein), respectively.

Because clocks are the manifestation of underlying forces and we do not yet possess a unified theory, we do not know a priori whether the ratio

$$\frac{\Delta s_{\rm E}}{\Delta s_{\rm a}} = \beta_{\rm a} \tag{8}$$

is constant. The lack of knowledge of the function β_a has so far been circumvented by adopting the strong equivalence principle^{3,4} (SEP) which assumes that

$$\beta_a = \text{constant} = 1$$
 (9)

implying that, for example, the value of the period of a planet should be independent of the clock used to measure it.

The SEP comprises two assumptions³: (1) that the weak equivalence principle (WEP) holds; and (2) that local gravitational and non-gravitational experiments are independent of where and when in the Universe they are performed. The requirement (9) refers to 'when in the Universe', since, due to the high degree of homogeniety observed in the Universe, we assume that β_a is only time dependent. Furthermore one experiment is not sufficient to test the SEP, at least two experiments are needed at two different times, because β_a can be normalized to unity at any one time; what is physically relevant is $\dot{\beta}_a$.

Tentative evidence of a violation of SEP

Using 8,249 lunar occultation measurements⁵, lunar radar ranging data^{6,7}, and dynamical determinations of the lunar periods⁸⁻¹⁰, it has been suggested that at the present epoch $\dot{\beta}_{\rm a}/\beta_{\rm a} \simeq 10^{-11}~\rm yr^{-1}$. However, as tidal forces make the Earth-Moon system less than an ideal laboratory for our purposes, it would be more satsisfactory to use radar ranging data to the inner planets Mercury, Venus and Mars. Using the available results, an upper limit $|\beta_{\rm a}/\beta_{\rm a}| \leqslant 10^{-10}~\rm yr^{-1}$ was set¹¹.

We stress here one aspect of the theoretical analysis. It may seem natural to use the standard Einstein equations with the simple alteration $G \rightarrow G(t) = G_0 + \dot{G}_0 \Delta t + \cdots$, whenever G appears. However, from Einstein equations it follows quite generally that for an isolated system GM = constant (refs 12, 13), where M is the total mass. (For any local system, this constraint holds regardless of cosmological expansion³.) The violation of this constraint can lead to serious errors. For example, because the period and distance to a planet are given by $P \sim J^3/(GM)^2$, $R \sim J^2/(GM)$, GM = constant implies R = 0 and $\dot{P} = 0$ and not $2\dot{R}/R = \dot{P}/P = -2\dot{G}/G$, as usually stated. This

argument shows that a value of \dot{G}/G cannot be extracted from the standard Einstein equations where by construction G always appears multiplied by M, the product being required to satisfy the constraint GM = constant.

For these reasons, we (in collaboration with R. Helling and P. J. Adams of JPL) have enlarged the system of dynamical equations (originally used by R. Helling) to make them compatible with a possible violation of the SEP. The Viking radar ranging data to Mars are now being analysed. The best fit solution will hopefully provide a reliable value for β_a .

Purpose and basic assumptions

Let us construct a theoretical framework that allows for an SEP violation in the form of a non-constant β_a . This allows for the presence of two fundamental systems of units in nature, which is necessary to explore consistently the implications of an SEP violation as well as to provide relations which can be subjected to observational test.

Gravitational or dynamical units (EU, where E stands for Einstein), are the units in which Einstein equations remain unchanged. These field equations contain the equations of motion that yield the clock equation (7), which is therefore taken to give the gravitational or dynamical unit of time (also called ephemeris time). In EU, G_E = constant by definition and so the total mass is also constant, since $G_E M_E$ is constant. In addition, from $T_{E,\nu}^{\mu\nu} = 0$ applied to a pressureless fluid, we find that the rest mass is also constant. Therefore, in general

$$G_{\rm E} = {\rm constant}, \qquad M_{\rm E} = {\rm constant}$$
 (10)

where M is either total or (macroscopic) rest masses. As the particle number N will be shown not to be constant, equation (10) does not mean that microscopic masses m_E are constant in EU. Other atomic quantities such as e and h, are also in principle not constant in EU.

Atomic units (subscript a) are the units in which the dynamical behaviour of an electromagnetic clock is governed by equation (4), which in turn implies that equation (5) is taken to be the atomic unit of time. Furthermore, in AU we have

$$e_a = \text{constant}, \quad m_a = \text{constant}, \quad h_a = \text{constant}$$
 (11)

The first two terms are the analogue of (10) for microphysics. (Actually, terms in equation (11) are contained in equations (4).) In analogy with what we said earlier, the second term of equation (11) does not imply that macroscopic masses M_a are constant in AU. In fact, they are not (see equation (25)); G_a is also not constant (see equation (18)).

Let us now consider the basic problem of determining the relation of the two 'preferred' systems of units. We introduce a language that describes any physical equation in a general system of units of which the two 'preferred' systems are special cases.

Physics in general units

Let us reconsider equation (1) and transform L_* to L_{**} ,

$$L_* \to L_{**} = \Omega^{-1}(x)L_* = (\Omega_*\Omega)^{-1}L \equiv \Omega_{**}^{-1}L$$

The quantity Ω_* has therefore power -1, as from equation (3), where Λ^* , Λ and Ω_* are replaced by Ω_{**} , Ω_* and Ω , respectively. Therefore, any quantity of the form $\Lambda^*\Omega_*^{\pi^{(\Lambda)}}$ has zero power under subsequent scale transformations:

$$\Lambda^*\Omega_*^{\pi(\Lambda)} = \Lambda^{**}\Omega_{**}^{\pi(\Lambda)}$$

As an example, we perform a further transformation of equation (6) to a g_{**} , F_{**} , J_{**} system. The final result can easily be seen to be of the same form as equation (6), with only double starred quantities in it. Therefore, equation (6) can be said to be written in general units.

Let us now define a fiducial system of units by $\Omega_* = 1$, and a general system of units by $\Omega_* = \beta$. Here, as in previous work¹⁴,

we choose EU as the fiducial system ($\beta_{\rm EU}=1$), so that to be consistent with equation (8), the AU system is defined by $\beta_{\rm AU}=\beta_{\rm a}$. Note that while β , defining a general system of units, has power -1, $\beta_{\rm a}$ is of power zero, because $\beta_{\rm a}=\beta_{\rm AU}/\beta_{\rm EU}$ is the same in all systems of units. Clearly the use of general units does not introduce any new physics.

The action

To deal with the problem of constructing the two clocks, we propose an action in general units, which as such must be of zero power,

$$I = \int \mu \beta^{2-g} ds + \frac{1}{16\pi} \int \beta^{2-g} F_{\alpha\gamma} F^{\alpha\gamma} \sqrt{g} dx^4$$

$$+ \int \beta^{2-g} e A_{\nu} u^{\nu} ds$$
(12)

where μ represents masses in general (microscopic or/and macroscopic) and where the relation between $F_{\mu\nu}\beta^{1-g/2}$ and $A_{\alpha}\beta^{1-g/2}$ is the usual one. It is easy to check that the power of I is zero. Because the dimensions of I are [M][L], the β^{2-g} factors are required for $\pi(I)=0$. The matter part of equation (12) is different from that proposed by Dirac¹⁵ which is a particular case of equation (12) if $\mu\beta^{1-g}=$ constant. In AU, and for microscopic masses, this implies g=1, because of equation (11). However, g=1 will be shown not to allow the two clocks to run at different rates. The relaxing of the restriction $\mu\beta^{1-g}=$ constant is why we can construct two clocks that run at different rates.

Macroscopic gravitational clock

Consider the periodic motion of a planet in the gravitational field of a star. From the first term in equation (12), we derive the following equations of motion in general units,

$$u_{:\gamma}^{\alpha}u^{\gamma} + \frac{(\mu\beta^{2-g}),\gamma}{(\mu\beta^{2-g})}\Delta^{\alpha\gamma} = 0$$
 (13)

where the metric $g_{\mu\nu}$ due to the star is given in general units by the Schwarschild metric times β^{-2} . As we are dealing with a macroscopic object, then

$$\mu = M$$
, $M_E = \beta^{1-g}M = \text{constant}$ (14)

where the second relation is the general law for mass transformation from Einstein units to general units, following from equation (3) with $\Lambda = M_E$, $\Lambda^* = M$, $\Omega_* = \beta$, $\pi(m) = 1 - g$. The last equality in equation (14) follows from equation (10). Equation (13), with equation (14), specializes to

EU:
$$u^{\alpha}_{;\gamma}u^{\gamma}=0;$$
 AU: $u^{\alpha}_{;\gamma}u^{\gamma}+\frac{\beta_{a,\gamma}}{\beta_a}\Delta^{\alpha\gamma}=0$ (15)

The solutions for the period P can be easily worked out. The results are

EU:
$$P_{\rm E} = \frac{2\pi J_{\rm E}^3}{(G_{\rm E}M_{\rm E})^2} = \text{constant};$$

AU: $P_{\rm a} = \beta_{\rm a}^{-1} P_{\rm E}$ (16)

Electromagnetic clock

The motion of an electron in the field of a proton $F^{\lambda\nu}$, is governed by the following two equations, derivable from equation (12)

$$(\sqrt{g}F^{\lambda\nu}\beta^{1-g/2})_{,\nu} = 4\pi \int e\beta^{1-g/2} \delta^4(x^\alpha - z^\alpha) dz^\lambda \equiv J^\lambda$$
 (17a)

$$u_{;\nu}^{\alpha}u^{\nu} + \frac{(\mu\beta^{2-g})_{,\nu}}{(\mu\beta^{2-g})}\Delta^{\alpha\nu} = \frac{e}{\mu}u^{\nu}F_{\nu}^{\alpha}$$
 (17b)

In equation (17b) we used $e\beta^{1-g/2}$ = constant, a constraint derivable from equation (17a) using the antisymmetry of $F_{\mu\nu}$. Let

us now specify equation (17) to AU and EU. In AU we require equation (17) to coincide with equation (4). In AU, $\beta = \beta_a$, and for a microscopic mass $\mu = m = m_a = \text{constant}$. The requirement can therefore be fulfilled only if

$$g = 2$$
, $\pi(m) = -1$, $G_a \beta_a^2 = \text{constant}$ (18)

To derive the last term of equation (18) we have used equation (3) with $\Lambda = G_E$, $\Lambda^* = G_a$, $\Omega_* = \beta_a$, as well as equation (10).

The corresponding solution for the period p_a in a local lorentzian coordinate frame is now given by equation (5), with the subscript a attached to all the quantities. Let us now consider EU, where

$$\beta = 1, \qquad \mu \equiv m_{\rm E} = m_{\rm a} \beta_{\rm a}^{\pi(m)},$$

$$e^2 = e_{\rm E}^2 = e_{\rm a}^2 \beta_{\rm a}^{1+\pi(m)}$$
(19)

where we have again used equation (3). Because g = 2, equation (17a) retains the same form as in AU, whereas equation (17b) becomes

EU:
$$u^{\alpha}_{;\nu}u^{\nu} - \frac{\beta_{a,\nu}}{\beta_a}\Delta^{\alpha\nu} = \frac{e_a}{m_a}\beta_a u^{\nu}F^{\alpha}_{\nu}$$
 (20)

Using a local lorentzian coordinate frame, the solution for the period $p_{\rm E}$ is found to be

$$p_{\rm E} = \beta_{\rm a} p_{\rm a}$$
, $(p_{\rm a} = {\rm constant, equation 5})$ (21)

To achieve the desired result we had to fix a gauge: a relation between β_a and G_a , equation (18). This is a welcome feature because until now, we had to consider g as a free parameter ^{16,17}. This no longer the case, as the theory now demands equation (18). Note that such a gauge was previously suggested in connection with the 3K black-body radiation ¹⁸.

We have proposed a lagrangian whose solution for the periods of gravitational (P) and atomic (p) clocks are

$$P_{\rm E} = \beta_{\rm a} P_{\rm a}$$
, $p_{\rm E} = \beta_{\rm a} p_{\rm a}$, $P_{\rm E}$, $p_{\rm a} = {\rm constant}$ (22)

or

$$\frac{p_a}{P_a} = \frac{p_E}{P_E} \sim \beta_a \tag{23}$$

namely: in either atomic or gravitational units, the ratio of the periods of the two clocks is not constant, provided β_a is not constant. We have therefore proved that, provided g=2, a framework exists which allows the two clocks to run at different rates.

We must stress that the extension of equation (17a) to a charged fluid must be written as $F^{\mu\nu}$; $_{\nu} = \tilde{J}^{\mu}$, where $\tilde{J}^{\mu} = enu^{\mu}f$, f being an undetermined function of β_a . Due to the antisymmetry of $F^{\mu\nu}$, it follows that \tilde{J}^{μ} ; $_{\mu} = 0$, which implies eNf = constant. Because for g = 2, e is constant in any units, equation (19); it then follows that $f \sim N^{-1}$. The Coulomb force now becomes $e^2N^2f^2/r^2$. The analogous gravitational force is GM^2/r^2 , with $GM^2 = G_EM_E^2 = \text{constant}$, a result valid for g = 2. The correspondence between macroscopic Coulomb's and Newton's laws is therefore preserved.

Weak equivalence principle

In achieving the result (23), the central part was played by the action (12) and the equations of motion ensuing from it. Since equation (13) is in general units, using g = 2, the fact that $m\beta^{1-g} = m_E = m_a\beta_a^{1-g} \sim \beta_a^{-1}$, and equation (14), we obtain

Microscopic bodies:
$$u^{\alpha}_{;\nu}u^{\nu} + \frac{\beta_{,\nu}}{\beta}\Delta^{\alpha\nu} = \frac{\beta_{a,\nu}}{\beta_{\alpha}}\Delta^{\alpha\nu}$$
 (24*a*)

Macroscopic bodies:
$$u^{\alpha}_{,\nu}u^{\nu} + \frac{\beta_{,\nu}}{\beta}\Delta^{\alpha\nu} = 0$$
 (24b)

which indicate that the two types of bodies do not follow the

same type of trajectories. Equations (24) are still in general

From the operational point of view, we are only interested in AU, so we limit our considerations to them. Equations (24) tell us that in AU, microscopic masses follow geodesics while macroscopic masses do not. In either case, however, masses do not enter the equations and the WEP is separately satisfied, in the sense that all macroscopic objects follow the same path as do all microscopic ones. The results of the Eotvos-Dicke-Braginskii experiments^{3,19}, showing that two (macroscopic) bars of Al and Au (or Al and Pt) follow the same path, are therefore in full agreement with equations (24), as the extra 'force' represented by β_a , is independent of the mass and it affects both bars equally. Thus, its effect cancels out in this type of

To test equations (24) one should follow in time the trajectories of an atom and of a planet, which is the procedure in the radar ranging experiments. In fact, one may think of atomic and gravitational clocks (the Earth-Moon system), as two 'objects' moving in space-time following two given trajectories. If, as time evolves, the two systems follow different types of trajectories, charting the time evolution of the macroscopic object with the reference provided by the microscopic object cannot yield constant results if the ratio of the proper lengths spanned by the two objects is not constant in time. Therefore equations (24) are an alternative way of interpreting the lunar and planetary data that stresses the difference in the two trajectories rather than the difference of the two clocks. These two ways of interpreting the data are equivalent, although the second one is the one almost exclusively referred to in this context.

Particle number non-conservation

The fact that only g = 2 is allowed has important consequences. In fact, since m_a and M_E are constant, it follows that (using equation (3) between AU and EU)

$$m_{\rm E} \sim \beta_{\rm a}^{1-g}, \qquad M_{\rm a} \sim \beta_{\rm a}^{g-1}, \qquad N \sim \beta_{\rm a}^{g-1}$$
 (25)

implying that N is no longer constant. To have a conserved N, we have to choose either β_a = constant, in which case the SEP is automatically satisfied or g = 1, which would also lead us to an SEP conserving framework. In fact, for g = 1 the left-hand side of equation (15) governing the macroscopic gravitational clock in AU would be identical to that of equation (17b) governing a microscopic electromagnetic clock in AU, thus leading to no difference between the two periods P_a and p_a , thus returning to the SEP. (The right-hand side of (17b) does not affect this statement because, due to spherical symmetry, it does not affect the angular momentum conservation law).

Equation (25) is the most important consequence of the SEP violation framework as it indicates that a violation of the SEP is intimately related to a violation of the particle number conservation law.

Gravitational constant

It is often stated that if atomic and gravitational clocks are different, the gravitational constant G must vary with atomic time. While the statement is not incorrect, it may give the impression that it adds some new fact. This is not the case. In fact, neither in the gravitational action of ref. (14) nor in the one presented here, is there an independent function G. One calls G the combination $G_E \beta^{-g} = G$, $G_E = \text{constant}$. But one does not introduce new physics, one simply lumps together a function β , a parameter g, and a constant G_E . That the physics is contained in β_a and not in G_a , is evident from the fact that the experiments on the Moon and the inner planets, yield directly β_a and not G_a , which is derived quantity, equation (18).

Cosmological meaning of β_a

The framework presented here while permitting the examination of the implications of an SEP violation, does not explain the physical mechanism behind it. In fact, β_a is treated here as an external quantity, much as viscosity is treated in classical fluid mechanics, where it enters as an external parameter whose evaluation requires a microscopic kinetic theory.

Although we do not offer a dynamics for β_a , it is important to stress that a dynamics of β_a can be either of a local or global nature. In the first case, β_a is regarded as a space-time field described by an action to be added to the total action; this would entail a coupling of β_a to local matter, with the result that even in EU, macroscopic gravity would no longer be described by standard Einstein equations, thus departing completely from our basic assumptions. A local approach was adopted by Brans and Dicke (BD). As several studies have indicated^{20,21}, Solar System experiments constrain, within the BD approach, the variability of β_a to some orders of magnitude below the value quoted previously. As there is no reason why we should arbitrarily restrict β_a to such low values, we find the local approach inadequate.

The value of β_a implying $\beta_a/\beta_a \sim H_0$ suggests that β_a is related to the structure of the Universe and that its dynamics is likely to be governed by topological rather than local space-time considerations. The SEP violation represents therefore a cosmological influence on local physics, in accord with Mach's principle. In contrast, accepting the SEP as an exact law of physics is equivalent to assuming that local physics is independent of the rest of the Universe.

Recent work on nucleosynthesis²² has indicated that, as expected, an SEP violation with a time scale of the order of the Hubble time cannot be extrapolated back to the radiation dominated era. Furthermore a dynamics for photons can be constructed²² independently of β_a : in particular, a very general argument has been found indicating that the photon number N_{s} , contrary to the particle number N_{s} , equation (25), is adiabatically conserved for any value of the parameter g. (The photon treatment presented in (ref. 16) is therefore valid only if g = 1).

The two previous results suggest that an SEP violation, if it exists, began to manifest itself only after the Universe entered the matter dominated era, before which β_a may have been constant.

Conclusions

The most important results of the present analysis are:

- Einstein field equations retain their standard form only in EU. In AU, they depend on β_a and their form is given by equation (2.34) of ref. 14.
- The trajectory of a macroscopic (many body) object is a geodesic in EU; in AU, β_a factors enter. The results are given in equation (24b).
- The trajectory of a microscopic (one body) object is a geodesic in AU; in EU, β_a factors enter, equation (24a).
- While in AU the description of a microscopic one body dynamics is unchanged, (this holds true even at the level of the Schrödinger equation), the description of a many-body situation is affected by β_a . In fact, the particle number $N \sim \beta_a$, equation (25). This in turn implies that macroscopic masses are such that $M_a \sim \beta_a$, $M_E \sim$ constant, Microscopic masses are such that $m_a \sim \text{constant}$, $m_E \sim \beta_a^{-1}$. Finally, the gravitational coupling G is such that $G_E = \text{constant}$, $G_a \sim \beta_a^-$

We thank Drs P. J. Adams, J. Anderson and J. Lodenquai for constructive criticism, and Ms Doris Smith for typing the manuscript.

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Protein dynamics investigated by the neutron diffraction-hydrogen exchange technique

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A new approach, using neutron diffraction and the hydrogen exchange (H/D) technique, has been used to study the extent and nature of the inherent conformational fluctuations in the protein, trypsin. The observed pattern of exchange was used to investigate systematic relationships between exchangeable sites and structural and chemical properties of the molecule. Results of this analysis indicate that hydrogen-bonding structure is the dominant factor governing rates of exchange. The model of conformational mobility which best explains the experimental findings involves a localized disruption of the secondary structure within different regions of the protein molecule, each limited in extent to the breaking of a small number of hydrogen bonds.

STRUCTURAL and chemical investigations have clearly demonstrated that, despite their high degree of hydrogen and hydrophobic bonding, proteins are far from rigid matrices of atoms and have certain component segments which exhibit considerable dynamic mobility¹⁻⁵. Because of their fundamental importance to the understanding of biological processes⁶⁻⁸ the dynamical properties of proteins have recently been the subject of intense study by a variety of methods. However, important issues remain concerning the effects of protein folding on the extent and nature of its dynamical motions.

I report here a study of the mobility characteristics of trypsin, a 223 amino acid protein, by coupling the hydrogen exchange (H/D) technique with neutron diffraction. Since its introduction by Linderstrom-Lang and his colleagues 9,10 , the H/D exchange method has been widely recognized to have great potential as a probe for protein conformational change (for detailed discussions of the potential applications of the H/D method to evaluating protein dynamics see refs 2, 11-14). H/D exchange has major advantages over other labelling techniques in that a deuteron has a negligible space requirement and has chemical properties nearly equivalent to those of the proton it replaces in the structure. Further, potentially labile sites are distributed throughout the molecule and therefore probe the dynamical properties of the whole structure.

Kinetic studies have shown that H to D exchange rates of peptide NH groups in a protein can differ by as much as 10 decades¹². It has been generally supposed that the groups which exchange at rapid rates similar to those characteristic of small molecules are those located on the surface of the protein and hence in direct contact with the solvent. Conversely, the groups which exchange much more slowly are assumed to be located in the interior of the protein and involved in internal hydrogen

It is generally accepted that in the conditions of this experiment (pH 7), the exchange reaction is catalysed by the presence of hydroxyl ion. However, little is known about other relationships between the exchange chemistry and the dynamical properties of proteins. For instance, the size of the solvent unit required to facilitate the exchange process is an open question, a situation which has led to the postulation of two distinct stereochemical models for exchange. One model, which will be referred to as the cooperative or local unfolding mechanism, is described by a transient, cooperative unfolding of a segment of secondary structure 11-13. Access to the exchangeable protons is assumed to be accomplished by the extrusion of the chain into the bulk solvent where the exchange reaction can proceed by normal water chemistry. In the second model, the penetration mechanism, it is assumed that the exchange reaction takes place, shielded completely from the bulk solvent, within the tightly packed interior of the protein. It is proposed that the necessary solvent molecules can be diffused through the protein to the interior sites via pathways opened by local atomic fluctuations¹⁴ or by mobile defects in the protein packing¹⁵.

A variation of local unfolding is introduced here and termed 'regional melting'. It differs from the mechanism described above in that exchange does not require the chain to be extruded into the bulk solvent. In this variant, the reconformation process is limited to the cooperative breaking of several hydrogen bonds, resulting in the formation of a solvent-filled cleft in the protein surface. The exchange is assumed to take place within the cleft if the cleft is sufficiently large to permit the solvent molecules to arrange themselves in a stereochemically productive orientation with respect to the exchange site. Although the solvent molecules within the clefts cannot be considered to have chemical properties identical to the bulk phase, they are assumed to be contiguous to the bulk solvent.

Unfortunately, previous H/D exchange experiments have been unable to relate exchange rates with specific groups or even regions of polypeptide chain, thus limiting the usefulness of exchange methods in elucidating the factors responsible for protein conformational mobility, and the mechanism of the exchange reaction.

Recently, the application of NMR spectroscopy ¹⁶⁻¹⁸ and chemical analysis ^{13,19,20} to H/D exchange has begun to provide data relating exchange rates to structural features. The present article reports on the application of neutron diffraction to this problem. Two factors make this technique particularly suitable for providing a direct and quantitative approach to the measurement of H/D exchange rates of specific sites. First, because it is a crystallographic technique, the precise location of each labile site in the well-ordered segments of the protein is known and can be examined directly in a neutron Fourier map. Second, because the amplitudes of hydrogen and deuterium scattering are of opposite signs (Fig. 1), the process of assigning a labile site as having either H or D character is quite a straightforward task, provided that the structure of the protein has been highly refined, as is the case for trypsin.

As will be seen from subsequent discussion, the goal of this study is not simply to obtain dynamical information about the trypsin molecule *per se*, but to use trypsin's structural units, its β -sheet and α -helix regions, its turns and loops, to investigate through H/D exchange how each type of unit reacts to the conformational mobility modes of the protein. Correlations between the exchangeability of a potentially labile site and four principal factors are examined: (1) the degree of local hydrogen bonding structure around the site, (2) its distance to the solvent, (3) the hydrophobic or hydrophilic character of adjacent side chains and (4) its observed vibrational motion (temperature factor).

Crystalline proteins as models for dynamic studies

It could be objected that, since diffraction methods are limited to the study of crystalline proteins, they might be expected to yield different results from studies of proteins in solution. The use of a crystalline system to model a dynamic phenomenon like conformational mobility is valid because a protein molecule in a crystal is highly solvated and has an environment very similar to that in solution²¹⁻²³. Thus, crystallographic studies have shown that a protein crystal can best be described as an ordered and open array of molecules held together by a relatively small number of intermolecular contact points. The interstitial region between molecules generally makes up about one-half of the total crystal volume24, and is comprised of a network of continuous channels filled with solvent having properties analogous to bulk water ^{23,25,26}. Because of the extent of unoccupied volume in the crystal, ions and substrates can readily diffuse through the solvent channels to interact with the protein. Analysis of the extent of the solvent network in the trypsin crystal (≈46% solvent by volume) revealed several channels having cavities as large as 18 Å across.

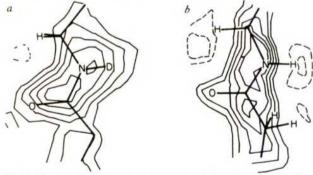


Fig. 1 Sections of a neutron density map taken in the plane of the peptide group. a, Fully exchanged peptide group, scattering length of $D=+6.7\times 10^{-13}$ cm; b, unexchanged peptide group, $H=-3.8\times 10^{-13}$ cm. In cases where partial exchange has taken place, the expected density at the H/D site is close to zero. This is because with H and D having scattering amplitudes of opposite sign, the composite scattering density tends to cancel in the Fourier summation. In the refinement, H/D sites were assigned a temperature factor based on that of the amide nitrogen, and occupancy factors alone were refined. In those regions of the structure with a high degree of thermal motion or local disorder, H/D ratios were not considered reliable and the affected residues are identified in Fig. 2 legend.

Given this structure, the protein molecules themselves would be expected to exhibit many of the chemical traits found in their fully solvated state. Particularly relevant to the present study are several investigations comparing the H/D exchange kinetics between crystalline and solvated protein systems^{27–30}. The exchange properties of solution and crystal systems were found to be very similar, implying that accurate exchange information can indeed be obtained from a crystalline protein system.

To determine whether or not lattice packing forces at contact areas between adjacent molecules in the crystal produced a measurable degree of inhibition of the exchange reaction, the peptides involved in the principal contact zones were identified and their degrees of exchange examined. All but one of the sites were found to be completely exchanged (the lack of exchange at this site is probably due to its being in a β -sheet structure and not a result of the contact), which demonstrated that such inhibition as might be encountered in the molecular contact regions was not significant for the reaction time of this experiment. However, this analysis does not exclude the possibility that some of the more extensive modes of motion might in some ways be impeded by the lattice forces.

Experimental procedures

A 5-mm3 crystal of monoisopropylphosphoryl (MIP) trypsin was soaked in a D₂O solution at pH 7 and 20 °C for ~1 yr before data collection was begun. (The MIP group is a small covalently bound inhibitor which does not affect the tertiary structure of the protein31.) Data to 1.8 Å resolution (14,500 reflections $> 3\sigma$) were collected on a two-dimensional positionsensitive detector system32 at the Brookhaven High Flux Beam Reactor. These data were processed by a method developed in this laboratory to deal specifically with the problems inherent in obtaining accurate intensity measurements from the weak neutron diffraction of protein crystals33. The initial phasing model was calculated by applying the appropriate neutron scattering lengths to the refined trypsin X-ray coordinates of Chambers and Stroud³⁴. Refinement of the structure was carried out by the restrained difference Fourier technique, which involves the determination of proper atomic shifts by the curvature-gradient method and then the re-idealization of the structural parameters by an energy minimization routine. Details of these experimental procedures are reported elsewhere³¹. The current trypsin model is highly restrained to ideal bond lengths and angles (r.m.s. error in bond lengths < 0.014 Å, bond angles $< 1.4^{\circ}$). After eight cycles of refinement the R factor is 19.2%.

The peaks produced in a neutron density map by sites having intermediate exchange ratios (between 15% D-85% H and 60% D-40% H) were found to give densities of a magnitude comparable with the general noise features of the trypsin map; therefore, prediction of relative exchange ratios between sites within this range cannot be made with statistical confidence. Accordingly, it is appropriate to subdivide the exchange ratios into three major categories: (1) unexchanged (0-15% D), (2) partially exchanged (15-60% D) and (3) fully exchanged (60-100% D). Limiting the number of categories in this manner will retain the informational content of the H/D data while guarding against over-interpretation of the relevance of small changes in exchange ratio.

Patterns of exchange

The degree of exchange of the amide hydrogens, as determined from the neutron diffraction analysis, is represented schematically in Fig. 2. Three-dimensional views of the main-chain atoms of the molecule represented in space filling and ball and stick form are shown in Fig. 3, which illustrates the exchange pattern as a function of the structure and folding of the protein. Sidechain groups were omitted to permit an unencumbered view of the intramolecular main-chain hydrogen bonding.

In summary, of the 215 exchangeable amide groups, 68% were found to be fully exchanged, 8% partially exchanged and 24% unexchanged. This corresponds to the cumulative result

of the exchange process throughout the entire span of the D_2O soaking of the crystal, because once a D replaces an H in the structure, any further exchange at the site is between Ds because of the low concentration of hydrogens in the soaking solution.

Although this experiment only distinguishes sites which exchange fast or slowly relative to the fixed time of reaction (1 yr at pH 7, 20 °C), the relatively low fraction of partially exchanged sites indicates a very substantial difference between the degree of protection provided by structural features of the protein to the unexchanged sites and that afforded the exchanged sites.

Inspection of Fig. 2 reveals that the unexchanged sites form a distinctive pattern whose most prominent feature is the clustering of unexchanged sites in regions corresponding to the B-sheet structures of the protein. The interior core of trypsin consists of two such structures, called '\(\beta\)-barrels', each consisting of six antiparallel strands laced together by a network of hydrogen bonds. In fact, 45 of 52 unexchanged hydrogens are found in the β -sheet structures. Closer examination of these regions shows that only 11 of the peptide groups involved in B-sheet hydrogen bonding are completely exchanged, and all of these are located at edges of sheet structures. Modelling studies performed by Salemme³⁵ show that extended β -sheet structures can withstand substantial deformation without disrupting their hydrogen bonding network. The present findings are consistent with this observation. Note, however, that the structural integrity of a β -sheet unit is not wholly a hydrogenbonding phenomenon, but also involves hydrophobic forces.

Significant data regarding the accessibility of sites within the tightly bonded β -sheet core come from the exchange behaviour of the hydroxyl group of the Ser 54 side chain. As illustrated in Fig. 4, which shows a segment of buried β -sheet (residues 43–45, 53–56), the Ser OH group forms hydrogen bonds to 43 O and 55 NH. Thus, it is embedded in the main β -sheet network and consequently has the same degree of protection as its neighbouring peptide groups. This hydroxyl group is found to be almost fully exchanged, while the amide proton of residue 55 is unexchanged, as are its neighbours.

The exchange of the OH group, which is considerably more reactive than peptide NH groups 11,12 , demonstrates that conformational fluctuations large enough to expose the β -sheet region to attack by the solvent do occur, but with insufficient probability for the slower peptide exchange to progress appreciably within the time frame of the experiment.

In addition to considering structural and bonding effects, it is important to examine and discuss several other parameters which might be expected to affect the exchange process, notably solvent accessibility, the chemical nature of side chains, and observed vibrational motion.

Effect of solvent proximity on rates of exchange

The degree of exchange of sites located near the surface of the protein molecule would generally be expected to be greater than that of sites buried in the interior. To test the magnitude of this effect, the distance of each peptide proton to the nearest bulk solvent interface was computed. Figure 5a plots this distance against the degree of exchange. The average distance between peptide sites and the protein surface was found to be $3.8 \, \text{Å}$.

As might be anticipated, >90% of unexchanged sites were located 4 Å or more from the surface. However, as many as 27% of the fully exchanged sites were also located 4 Å or deeper. Because access to such sites would presumably require rather substantial conformational fluctuations, the 20 exchanged sites located 5 Å or deeper were examined to determine whether they were near one of the small clusters of water bound in cavities inside the molecule. Nine of these sites were, in fact, located adjacent to one of several interior water molecules. Thus, if the distance to the solvent interface is redefined to include interior water cavities, the average distance

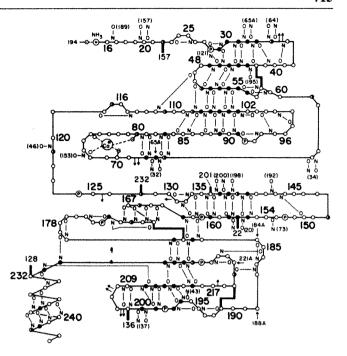


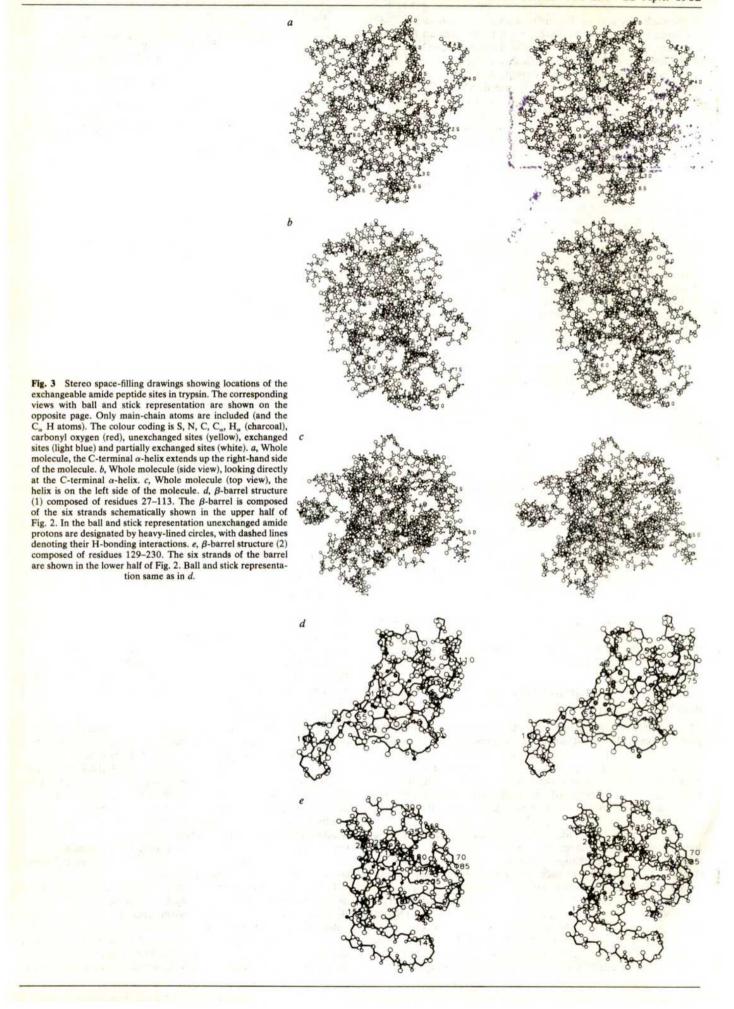
Fig. 2 Schematic representation of H/D exchange at each amide peptide site in the trypsin molecule. Key: ○, full exchange; ④, partial exchange; ♠, unexchanged; ⊕, proline; ↓, sequence insertions; ↑, deletions (based on the chymotrypsinogen numbering scheme); ⊝, carboxylate side chains. Peptide NH and carbonyl O atoms are shown when H-bonded. Disulphide bridges are indicated by broad lines. Because of the effects of thermal motion or local disorder, the H/D exchange information for residues 60–62, 110–111 and 147–150 is not reliable. (Figure design adapted form Birktoft and Blow⁴⁶.)

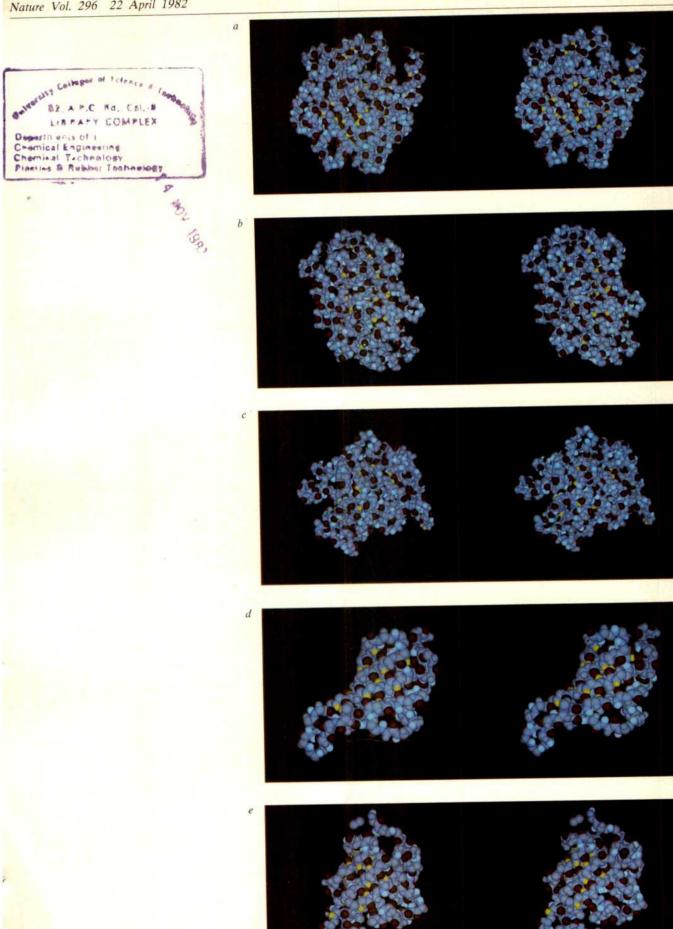
of these sites from solvent decreases by 2.65 Å (only two sites remain as far as 5 Å from solvent) and the overall average distance of all peptide protons in the molecules from solvent decreases by 1.1 Å to 2.7 Å.

Recent investigations have shown that trypsin is not a unique protein in having a significant number of interior water cavities (I. D. Kuntz, personal communication). The finding that the water molecules residing in the cavities are fully exchanged for D₂O suggests that free solvent exchange occurs between the bulk and interior solvent regions. Figure 6, which shows a low-resolution (5 Å) electron density model of trypsin, gives some idea of the extent of these cavities. Note that, because of the limited resolution of the data and the density contour level used in the construction of the model, the actual sizes of the cavities are exaggerated. Even so, the 5 Å density model still presents a very instructive picture regarding the extent of the cavity network in the trypsin molecule. (The entrances to the pockets holding the interior water molecules are marked in Fig. 6.) Further, a majority of the holes pictured in the 5 Å model were found to coincide with the location of the set of cavities determined from the refined 1.8 Å structure.

In several cases these water cavities are sealed off from direct contact with bulk solvent by only one or several side-chain interactions; the reorientation of the side chains to accommodate interior-bulk solvent contact would presumably be of relatively low energy compared with structural fluctuations which break main-chain hydrogen bonds. Hence, it is plausible that structural fluctuations of the groups surrounding these internal cavities might represent natural low-energy pathways for solvent access and 'regional melting'.

It was noted above that a strong correlation exists between non-exchanged peptides and hydrogen-bonding sites in the β -sheet structures. As the β -structures are located deeper within the protein (3.8 Å) than the remaining sections (2.3 Å), an effort was made to separate out the effects of structure and depth. The average depth of the 11 fully exchanged sites located in the β -sheet structures is 4.0 Å, which corresponds closely





to the average depth (3.8 Å) of the total β -sheet population and implies that distance to the solvent is not a primary factor in determining exchange characteristics of β -structures.

In the non- β -sheet sites, the 133 fully exchanged sites were located at an average distance of 2.1 Å from the solvent, while the 7 unexchanged sites were at 4.3 Å. This suggests that the depth effect may be a real one, but the number of unexchanged sites is too small to provide confidence in the result. Note that even after the interior water cavities are taken into account, many sites 3 and 4 Å from the solvent interface are fully exchanged. This would imply that the molecule has sufficient internal mobility to provide access to sites when they are not immobilized by secondary structures.

A graphic example of the dominant role of secondary structure on exchange characteristics is illustrated in Fig. 7, which shows a region of β -sheet containing an occluded water molecule. In the structure, a pair of hydrogen bonds is formed between peptides 215 and 227; the amide hydrogens are unexchanged in both peptide groups. There is a well-ordered water molecule perpendicular to the plane of the sheet which hydrogen bonds to both carbonyl oxygens and to Ser 190 O, (not shown). The clear implication is that an amide peptide group can participate with water (in this case D2O) in a hydrogen bond to the same acceptor ligand and yet remain unexchanged. (This is not an isolated case, since similar arrangements have been observed in five places in the trypsin molecule.) In the above instances there is clearly solvent access to peptide sites which nevertheless remain unexchanged, indicating that structural (bonding) effects dominate over the effect of solvent accessibility.

Neighbouring side-chain effects

It has been suggested that the different chemical characteristics of side chains might, by inductive effects³⁶ or by changing the

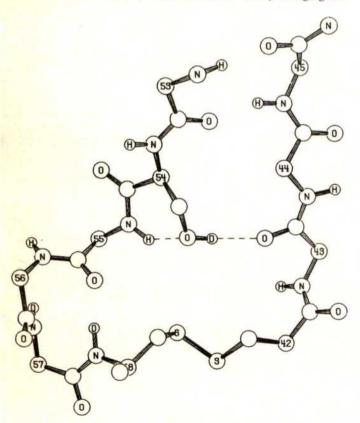


Fig. 4 Section of β -sheet structure residues 43–45 and 53–56 showing main-chain atoms (and the side chain of Ser 54). Because of a bulge in the sheet, the H-bond interaction between 43 O and 55 NH cannot be made directly. The bonding structure of the sheet is maintained (dashed lines) through the mediation of the side chain of Ser 54. The hydroxyl proton of the serine is almost fully exchanged for a D, yet the amide proton of Ala 55 to which the hydroxyl oxygen is H-bonded remains unexchanged.

local water chemistry ^{37–39} around the neighbouring peptide site, significantly affect the rate of H/D exchange. The importance of inductive effects was verified by Molday et al. ³⁶ who systematically evaluated the nearest-neighbour side-chain effects using small model peptides and derived a set of empirical rules which could predict rates of exchange in random order peptides from their amino acid sequence. Most of the hydrophobic and hydrophilic side-chain effects were found to span a 10-fold range. Other studies have shown that exchange in model polyamides is slowed by the presence of bulky apolar groups, even though the peptide nitrogen itself was not involved in hydrogen bonding ^{39–41}. In the light of these investigations, it is important to ascertain whether or not patterns of exchange in the trypsin molecule are also affected by the nature of the associated side chains.

The observed character of these near-neighbour effects³⁶ might suggest that the presence of a hydrophobic side chain would make the adjacent nitrogen more resistent to exchange. In trypsin, the groups alanine, leucine, isoleucine, methionine, phenylalanine and valine account for 65 of the 215 potentially exchangeable sites. Of these, 44% were found to be fully exchanged, 8% partially exchanged and 48% unexchanged. Of the hydrophilic groups—arginine, asparagine, aspartic acid, glutamine, glutamic acid, histidine and lysine—which account for 53 sites, 79% were exchanged, 8% partially exchanged and 13% unexchanged.

Comparison of above values with the average for the entire molecule (68% fully exchanged, 8% partially exchanged and 24% unexchanged) indicates a strong correlation between the degree of exchange and the polarity of the associated side chain. However, before concluding that this correlation is significant, it is necessary to determine whether this effect is independent of the structural correlation previously observed between β -sheet structures and exchange character.

There are 59 sites involved in the two six-stranded β -sheet structures. Of these, 29 are hydrophobic, while only 11 are hydrophilic. (Such hydrophobic/hydrophilic ratios are typical of β -structures of most globular proteins⁴² and it is generally assumed that the high concentration of hydrophobic groups contributes to the unusual stability of these structures.) These β -sheet sites account for all but seven unexchanged groups. Thus, when the members of the β -sheet structures are removed from the hydrophobic population, a large percentage of the remaining groups are fully exchanged (72% fully exchanged, 8% partially exchanged and only 20% unexchanged). Conversely, of the 11 hydrophilic groups which participate in β -sheet structures, 27% are fully exchanged, 9% partially exchanged and 64% unexchanged.

The above analysis suggests that in the conditions of the experiment (1 yr soaking), any effect of side-chain character which might either promote or inhibit exchange is dominated by the more powerful effects of structure and the hydrogen-bonding character of the sites.

Temperature factor correlations

Investigators have attempted to gain insight into the phenomenon of dynamic mobility by using temperature factor data from detailed X-ray analyses of several proteins 3.4.43. The attraction of this method is that it identifies specific groups in the protein structure whose locations show positional variation due to thermal motion. However, the method has several limitations. Protein dynamics as described by temperature factor correlations involve small atomic displacements (of the order of 0.5-1.5 Å), and thus represent as a class rather low-energy conformational fluctuations. Further, at the resolution level of normal protein structure analysis (that is, below atomic resolution), accurate individual temperature factor values (B factors) are difficult to obtain. Local trends of group B factors offer a considerably more reliable measure of motion and, therefore, during the refinement procedure, B factors are usually restrained in some fashion to be similar to those of their near neighbours.

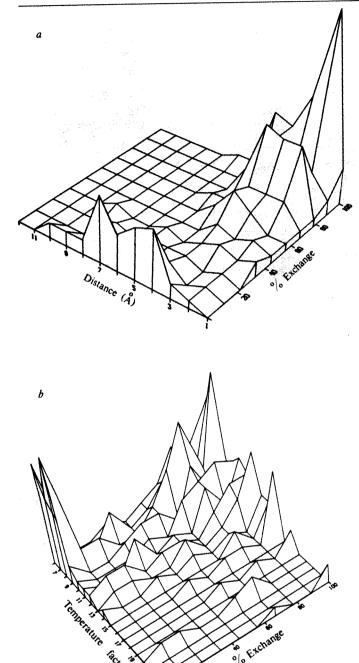


Fig. 5 a, Exchange ratios (0-100%D) of the peptide NH sites plotted as a function of their distance to the bulk solvent. Internal water cavities were not considered in the analysis. The molecular envelope was calculated using an atomic radius of 1.4 Å for all atom types including hydrogen. A radius of this size, when applied to hydrogen, overestimates the spatial volumes of most groups and therefore tends to extend the envelope outside the bounds of the molecule defined by the coordinates of the static structure. However, it was felt that in this type of analysis it was important to enlarge the molecular envelope slightly to take into account the small vibrational motions and positional disorder of the surface residues, effects which also increase slightly the molecular boundary. Thus, the resulting site to solvent distances are defined here to reflect a maximum distance, that is, a distance of 1 Å represents a true distance of 0-1 Å to solvent; a distance of 2 Å is 1-2 Å and so forth. Note that in most instances solvent proximity, as defined here (shortest distance to the solvent interface), would not be expected to correlate with solvent accessibility exactly because the low energy pathway by which solvent gains access to a site does not necessarily correspond to the same pathway which defines the shortest distance. b, Plot of exchange ratios of the peptide NH sites as a function of their experimentally determined temperature factors. Exchange categories are partitioned as follows: (1) unexchanged (0-15% D), (2) partially exchanged (15-60% D), (3) fully exchanged (60-100% D). Heights of the peaks correspond to the number of sites at each occupancy grouping. Highest peak in a (which is distance 2 Å and 100% D) represents 25 sites, in b a temperature factor of 8 at 0% D represents 10 sites.

Sternberg et al.³ attempted to use thermal factor parameters to describe correlated rigid body motilities of extensive segments of polypeptide chain, applying a sophisticated mathematical treatment to assess the modes of intramolecular motion (so called hinge-bending motions) between the two major globular lobes in lysozyme. From their analysis they conclude that temperature factor information offers only weak evidence for any hinge-bending motions. This suggests that if the coupled modes of movement between the two lobes exist in lysozyme they are structural fluctuations of the high-energy, short-lived type.

The conclusions of Sternberg et $al.^3$ are corroborated by the findings of the present neutron analysis. Figure 5b shows the correlation between individual amide peptide temperature factors and H/D exchange ratios. As expected, the unexchanged amide groups are distributed in a narrow range at low temperature factors, indicating that they are predominantly located in interior, highly structured regions of the protein. It is somewhat surprising, perhaps, to find that many of the fully exchanged sites are also distributed near the low end of the temperature factor scale. This suggests that they, too, are highly ordered, at least on a crystallographic time scale, but must become sufficiently exposed to solvent as a function of the general breathing modes of the protein to promote exchange.

Because many exchanged interior sites have B-factor values comparable with those of the unexchanged sites, I conclude that no distinct correlation exists between the potential exchange of an interior site and its observed thermal motion. This finding has an important implication with respect to the use of crystallographically determined temperature factors to describe protein motions. Although these parameters have been shown to be of value in identifying local dynamic trends involving small atomic displacements, they appear to contain no systematic information on the characteristics of the larger-scale breathing modes of this protein.

Concluding remarks

It has been reported that the variation in the rate of H/D exchange of peptide groups in a protein molecule ranges over as much as 10 decades¹². The conditions of the experiment reported here were apparently such as to exchange all except those sites which were especially well protected by the protein structure. Clearly, an important future study would involve a comparative analysis of a crystal soaked for a shorter time to identify those sites which exchange slowly on an absolute scale, but rapidly enough to be fully exchanged in the conditions used here.

In summary, it appears that essentially all the sites in which the peptide hydrogens are bonded directly to water molecules—either in the bulk solvent regions or in interior clusters—are fully exchanged. No significant inhibition of peptide exchange was apparent at molecular contact points in the crystal, at hydrophobic side chains or as a function of degree of vibrational motion of the site, except when any of these also corresponded to hydrogen-bonded structures. Further, most of the sites in which the peptide hydrogens are involved in isolated bonds to either side-chain groups or main-chain carbonyls are fully or partially exchanged. Finally, those sites in the β -sheet structures which are not only hydrogen bonded to main-chain carbonyls, but are also flanked by similarly bound peptides, are to a very large extent unexchanged.

In the two short segments of α -helix in the trypsin molecule, the peptides are almost wholly exchanged. The only fully unexchanged sites, Ile 238 and Ile 242, are located along the interface between the helix and two strands of β -sheet. The spatial relationship between these groups and the sheet can be seen in Fig. 3a. (As pictured, the helix is located on the right side of the molecule with the amide protons of 238 and 242 shown in yellow.) The finding that the exchange properties of the helix peptide nitrogens differ substantially (apparently depending on whether or not hydrophobic intramolecular contacts are made) indicates that such conformational fluctuations

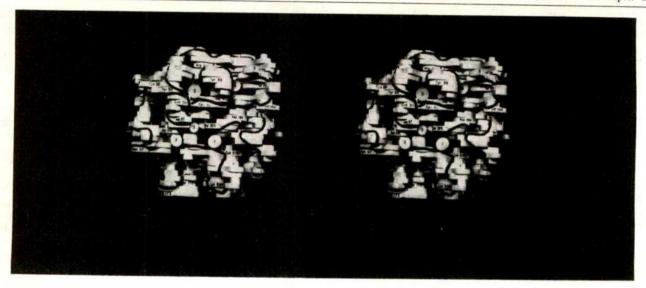


Fig. 6 5 Å density model of trypsin. Water cavity pockets are numbered. Cavity number 1 is the entrance to the substrate binding pocket.

as may be responsible for exposing the groups to solvent probably involve the breaking of hydrogen bonds only adjacent to the exchanged sites rather than the cooperative unfolding of the full helix unit. If such an unfolding process were to occur, it would be difficult to explain how these two particular sites remain unexchanged (especially in light of the fact that they both fit a distinctive pattern with respect to their packing against the β -sheet).

Figure 7 illustrated the important point that solvent accessibility alone is not a sufficient condition for exchange. The finding that rates of exchange are highly correlated to the structural features of the protein, especially hydrogen bonding, is consistent with the hypothesis that the segments of a protein molecule continually undergo local conformational fluctuations in which interior sites occasionally become aligned in chemically reactive orientations with solvent molecules. This mechanism predicts that the relative exchange rate constants would be dependent on the equilibrium concentrations of the unfolded species, which in turn would be a function of the energy required to overcome the local internal bonding of the folded structure. However, there is no evidence that extensive reconformation of the polypeptide chain is necessary to expose even the well buried exchanged sites. The finding that the exchangeable sites are partitioned almost exclusively into just two categories (fully exchanged and fully unexchanged, with relatively few sites being partially exchanged), implies that significantly different energies are required to overcome the internal bonding within the various domains in the molecule. Similar findings have been obtained through other experimental techniques 44,45.

The fact that full exchange occurs at regions of direct contact of adjacent molecules in the crystal, where gross motion is certainly minimized, indicates that outside the β -sheet structures relatively limited displacements of the structure suffice to provide the necessary access to enable exchange to proceed. This is also borne out by the complete exchange of internally occluded water molecules. Although it seems likely that solvent cavities provide natural pathways for access to some of the deeply buried sites in trypsin, the principal results of this experiment are inconsistent with a mechanism based on the premise that depth of burial of a site is a primary factor governing its rate of exchange. The most likely model to explain the observed exchange behaviour seems to be one involving localized conformational mobility, where the most probable fluctuations are those characterized by a degree of 'regional melting' usually limited in extent to the breaking of only a small number of hydrogen bonds. Further investigations aimed at resolving issues concerning the relationships of exchange chemistry to

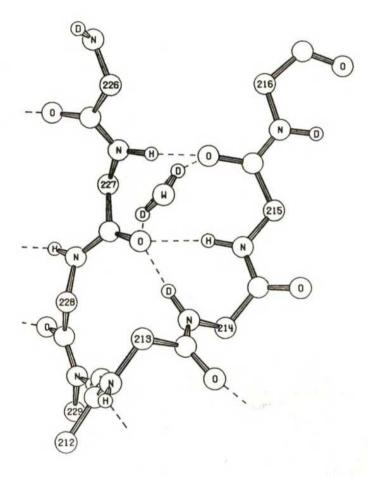


Fig. 7 Section of β -sheet showing the H-bond interaction between 227 NH-215 O, and 227 O-215 NH. An ordered D₂O molecule shares this H-bond, yet the peptide NH protons are unexchanged. The locations of the D atoms on the D₂O molecule were determined from the neutron density map, to indicate how the D₂O was oriented relative to the coordination ligands (215 O and 227 O). Note that the 214 amide is fully exchanged. Calculations indicate that the 214 and 215 amide protons are equally close to the solvent interface. Their respective hydrogen bond strengths are quite different. The 227 O-215 N hydrogen bond is classified as normal (2.9 Å), whereas the 227 O-214 N distance is quite long (3.5 Å), indicating a weak interaction.

protein dynamics should profit by the application of modelling studies using the exchange information reported here as a data

I thank Dr S. Spencer for his assistance with all aspects of the trypsin analysis, J. Shpungin for technical help and Drs A.

Recieved 28 October 1981; accepted 15 February 1982.

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The formation of galactic haloes in the neutrino-adiabatic theory

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Recent evidence of a finite rest mass for the neutrino has led to a revival of the idea that these particles may provide the unseen but dynamically indicated matter on cosmological scales of galactic halo and larger size. In the early Universe, the free streaming of collisionless neutrinos is seen to damp out perturbations less than of the order of tens of Mpc long1 numerical simulation of the clustering of collisionless particles in a universe with this type of perturbation spectrum⁶ has led to the formulation of a cell structure with large voids. Many observations^{7,8} support the existence of such a structure on scales of tens of Mpc. However, collapse of perturbations of this size leads to velocities of the order of 1,000 km s⁻¹, and as the neutrinos presumably cannot cool, this seems to be too large for their inclusion in galactic haloes, which have much smaller velocity dispersions. Thus it has been suggested that neutrinos cannot cluster in galactic haloes without some means to 'save' smaller-scale perturbations^{3,4,9}. Furthermore, if neutrinos of mass ~30 eV form galactic haloes, the phase space density must be very close to the primordial one 10—and phase mixing is often associated with collapse. Although the average value of the velocity dispersion may be reduced if the perturbations are anisotropic11, a disparity still remains. I now present a numerical simulation which shows that if the initial perturbations are sufficiently anisotropic, the collapse of very large perturbations of collisionless particles leads to a condensation of particles with low velocity dispersion and high phase-space density, which may easily fragment.

A series of numerical simulations were initiated to study the collapse of large-scale perturbations of massive neutrinos. The technique used was the "cloud in cell" method¹², which accurately models collective effects while suppressing two-body effects (such as the large-angle gravitational scattering of one neutrino by another), which are not important for neutrino clustering. The gravitational potential was derived using a Fast Fourier Transform method, with periodic boundary conditions, in co-moving coordinates6. I began with a study of the planesymmetric case, an approximation to highly anisotropic collapse, because anisotropy may increase during collapse¹

A high-resolution one-dimensional simulation, to be published elsewhere 14, showed that the central region of the phase space (see Fig. 1) is dominated by a high phase-space density, low velocity dispersion condensation. A sinusoidal perturbation of 100 Mpc wavelength was chosen for the simulation so as to test the hypothesis severely. The 100 Mpc field was divided in successive simulations into 10², 10³ and 10⁴ grids, onto which were placed 10³, 10⁴ and 10⁴ particles respectively. Changing the resolution from slightly larger to much smaller than the condensation had no effect on the conclusion, apart from low-level noise introduced in the third simulation by the smaller number of clouds per cell. Imposition of even highly asymmetric initial perturbations resulted in an asymmetric condensation, with a small net momentum, but still with the crucial high phase-space density and low velocity dispersion.

For a sinusoidal initial perturbation of amplitude $\delta \rho / \rho \sim 10^{-3}$ at $z = 10^4$ and a wavelength of 100 Mpc, in a universe of Hubble constant 75 km s⁻¹ Mpc⁻¹ and Ω (density relative to critical density) of 1.07, about 12% of the (10⁴) particles were bound in the central Mpc, with velocities < 200 km s⁻¹. Furthermore, the condensation was found to have a phase-space density very close to that of the primordial distribution.

A two-dimensional code was developed, using a 100×100 grid field scaled to 1 Mpc per grid. This code was found to conserve energy to 1.7% or better (a considerable improvement over past calculations of this type⁶), depending on choice of time step; to reverse itself from a collapsed state to the uniform distribution; and to behave like an analytical solution for the

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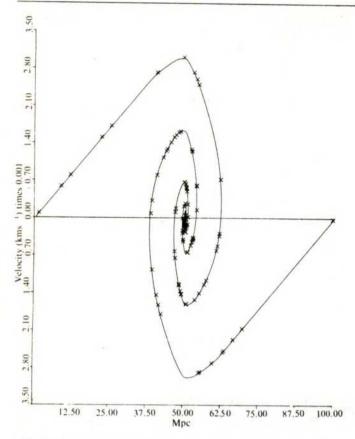


Fig. 1 A plot of the phase space of the one-dimensional simulation at Z=0. A solid line connects the particles, and a cross is drawn at the position of every one-hundredth test particle. Note the condensation of particles to the central Mpc.

case of uniform spherical symmetric perturbations. It contained two populations of particles: those given no initial velocity and a test population with thermal velocities appropriate to 30 eV neutrinos.

The result of collapse to Z=0 is seen in Fig. 2. A central slab is visible in the plot in which the darkness of a grid is proportional to its mass density. An $x-V_x$ projection of this result duplicates Fig. 1. The central region would be the predicted site of galaxy formation in the adiabatic theory, as the 'normal' matter undergoes collision and shocking. Somehow the collisionless neutrinos must cluster around these galaxies.

Figure 3 shows the result of assigning only 1% of the mass to 'protogalaxy grids' at Z=5, the moment of pancaking of the neutrinos. The pattern again is that of the distribution of test particles, which are strongly clumped around the protogalaxies. The same clumping appeared if the mass was slowly assigned to protogalaxies from Z=5 to 4. In this case, it was found that 7% of the test particles were bound within the protogalaxy grids and had velocities $<200 \,\mathrm{km \, s^{-1}}$. If the protogalaxies are chosen to be of smaller mass, distributed more evenly, the amount of material 'captured' increases because fewer particles acquire velocities greater than the $200 \,\mathrm{km \, s^{-1}}$ limit. Very small perturbations are sufficient to fragment the neutrino pancake.

Figures 2 and 3 are not to the same gray scale—black is adjusted to be the grid of highest density. The dark grids in Fig. 3 are of the same density as the local group, if it is bound.

Therefore sufficiently anisotropic perturbations will permit massive neutrinos to cluster in regions of low velocity dispersion, high mass density and high phase-space density. P. J. E. Peebles (personal communication) has pointed out that the central mass density of these regions must be much larger than the average shown here. My simulation cannot accurately model the dynamics inside a grid. It has been shown 13, however, that

a condensing protogalaxy can capture a significant number of neutrinos, resulting in a density enhancement in a central region, so that the neutrino density becomes comparable with the baryon density, which is demanded by flat galactic rotation curves.

Therefore the collapse of large-scale perturbations of collisionless particles results in a condensation of particles of low velocity dispersion and high phase-space density. This con-

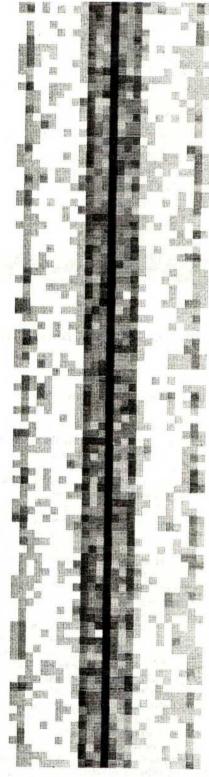


Fig. 2 A plot of the density of the two-dimensional simulation, at Z=0, given the same initial conditions as in the one-dimensional simulation. A small population of particles exists outside the slab, too light to be seen in this rendering.

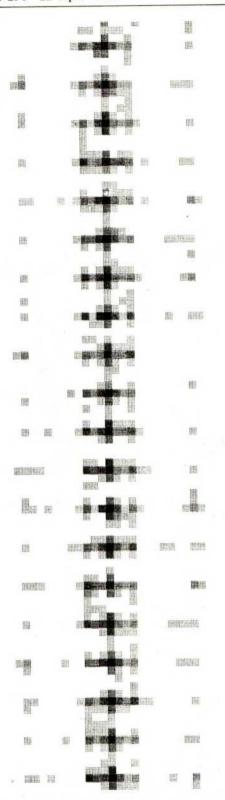


Fig. 3 A plot of the same initial conditions as in Fig. 2, if 1% of the mass is divided between 'protogalaxies' in the caustic surface. The clustering of the test particles is pronounced.

densation is subject to fragmentation by introduction of small 'protogalaxy' mass perturbations in the central plane, and is available for capture by collapsing protogalaxies.

Since many neutrinos would lie beyond the region of galaxy clustering and the Hubble expansion rate may be highly anisotropic, estimates of Ω from observations of the deceleration of galaxies may be incorrect. Therefore the clustering of massive neutrinos to account for the 'hidden mass' on all scales

is not in conflict with simple assumptions, provided that the initial perturbations are sufficiently anisotropic.

I thank D. W. Sciama for support, encouragement and training, S. Weinberg for financial support, and the Department of Physics, University of Texas at Austin for allowing the large amount of computer time needed to conduct this study. A conversation with J. Binney was helpful in planning the study, and N. Sharp assisted in computing.

Received 23 November 1981; accepted 17 February 1982.

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High-energy γ -ray light curve of PSR0531+21

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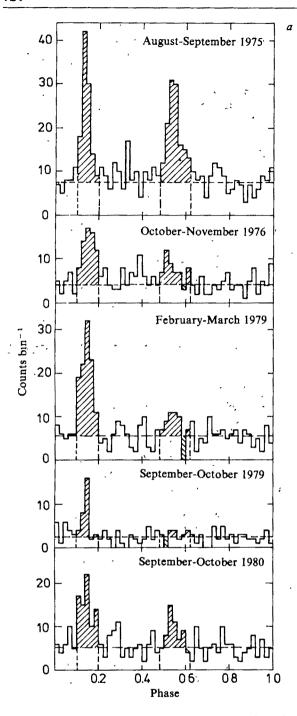
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The Crab pulsar (PSR0531+21) was one of the earliest identified sources of high-energy γ radiation 1-6. As such it was selected as the first object of detailed study by ESA's γ-ray astronomy satellite COS B, launched in August 1975. The experiment and mission have been described elsewhere7. In the first 6 yr of operation COS B has made five observations (each of 30-40 days duration) in which PSR0531+21 was within 15° of the centre of the field of view. The first of these confirmed the result of the SAS 2 experiment that the γ -ray light curve for energies above a few tens of MeV consisted essentially of two equally strong peaks but the later measurements found a decrease in the strength of the second peak relative to the first. No change was observed in the 2-12 keV X-ray light curve simultaneously measured by COS B. The total data from the five observations contain the first evidence of interpulse emission between the two peaks in the energy range 50-3,000 MeV.

The epochs and observing conditions are summarized in Table 1. The accuracy of the on-board clock and the precision of reconstitution of the position of the satellite in its orbit as a function of time permit the measured arrival times of the γ-ray photons to be transformed to the Solar System barycentre (SSB) with an uncertainty of 0.5 ms. In making this transformation the pulsar position measured at radio frequencies8 was used.



The phase ϕ of a γ -ray event with SSB arrival time T is calculated from the frequency f and its derivatives f, f at epoch T_0 according to

$$\phi = \phi_0 + \Delta T f + \frac{1}{2} \Delta T^2 f + \frac{1}{6} \Delta T^3 \tilde{f}$$

where

$$\Delta T = T - T_0$$

The values of these parameters used to analyse the present-observations are given in Table 1. For the first three observations radio parameters were available (ref. 9 and personal communications from V. Boriakoff and D. Ferguson, and J. M. Rankin). For observation 54 use was made of the COS B 'pulsar synchronizer,' a proportional counter sensitive to 2-12 keV X rays¹², the arrival times of which are measured with the same precision as for the γ rays. As there are many more X-ray photons than γ rays, it was possible to determine the pulsar frequency and its first derivative by testing trial light curves for values of these parameters scanned around the extrapolations from earlier epochs. The values adopted are those which gave

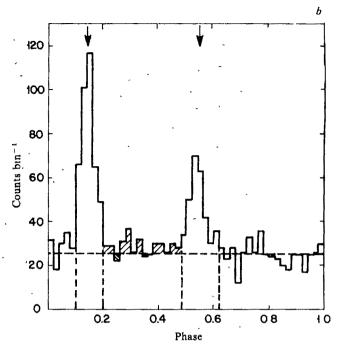


Fig. 1 a, γ -ray light curves of PSR0531+21 at the epochs indicated. Events were selected by energy E and arrival direction θ according to $50 < E < 150 \,\text{MeV}$ and $\theta < 7^\circ$ or $150 < E < 3,000 \,\text{MeV}$ and $\theta < 4^\circ$. The shaded areas indicate the phase intervals of the two pulses and the horizontal broken lines show the level of the background. b, γ -ray light curve obtained by summing the individual curves. The horizontal broken line shows the background level and the shaded area indicates the interpulse emission. The two arrows indicate the phases of the radio peaks.

the X-ray light curve having the highest χ^2 . (For a discussion of the method see ref. 11.) This approach could not be used for observation 44 because the pulsar was outside the field of view of the X-ray detector. Instead the arrival times of the γ rays themselves were used in a parameter scan. The features of the resulting light curve may therefore be over-emphasized but this observation is trivial in the final conclusions.

High-quality y-ray events were selected from the data by applying established and calibrated criteria to the sparkchamber pictures and counter pulse-height measurements. The energy spectra of the pulsar γ rays¹² and of the background radiation are such that the best signal-to-noise ratio is obtained within the energy range 50-3,000 MeV. To take account of the variation with energy of the instrument response (effective sensitive area and angular resolution) the data were analysed in two separate energy ranges, 50-150 MeV and 150-3,000 MeV. Within each range the signal-to-noise ratio could be further optimized by selecting events for which the measured arrival direction fell within a limiting cone centred on the direction of the pulsar. The half angle of the optimum cone was found to be 7° in the lower energy range and 4° in the upper. Figure 1a shows the y-ray light curve for each observation obtained by summing the respective curves for the two ranges. These curves show the actual numbers of photons recorded in each phase bin. Absolute fluxes are not presented. because their accurate determination depends on the analysis of the temporal development of the instrument's sensitivity over the five years and this analysis is still in progress.

Figure 1a shows clearly that for the later observations the second pulse in the light curve is weaker relative to the first than it is in the first observation. For a more quantitative study phase boundaries were assigned to the two peaks, namely 0.10-0.20 and 0.48-0.62 respectively. A background level, above which 'pulsed counts' were measured, was derived as the mean level in the phase interval 0.62-0.10. This background includes not only the instrumental background, the general

Table 1 COS B observations of PSR0531+21					
Observation no.	1	14	39	44	54
Date .	17 August– 17 September 1975	30 September– 2 November 1976	22 February– 3 Aprıl 1979	29 August– 10 October 1979	4 September- 17 October 1980
Pointing direction (l, b) Aspect angle of pulsar	184°, –6° 1°	195°, +4° 15°	190°, 0° 8°	172°, -12° 14°	188°, −3° 5°
Parameters of analysis	· ·				
Epoch	2442647.0	2442982 0625	2443946.442	2444128.5	2444508.5
$f(\mathbf{s}^{-1})$	30.13744554816328	30.12634886	30.094467179	30.088458052	30.075925066
$f(10^{-10} \mathrm{s}^{-2})$	-3.8348446531	-3.8310414	-3.82125043	-3.81956	-3.81527838
$f(10^{-20} \text{s}^{-3})$	1.1413101714	0.3956	1.225	1.224	1.22
Offset ϕ_0	0.352344	0.376843	0 958065	0.86	0.85
Pulse-strength ratio P_2/P_1		•			
50-150 MeV	1.34 ± 0.38	0.33 ± 0.27	0.36 ± 0.18	*	0.43 ± 0.32
150-3,000 MeV	0.74 ± 0.21	0.57 ± 0.31	0.35 ± 0.18	0.38 ± 0.27	0.31 ± 0.18
50-3,000 MeV	1.04 ± 0.20	0.49 ± 0.20	0.36 ± 0.12	0.48 ± 0.31	0.37 ± 0.17

^{*} Not determined due to insufficient statistics.

galactic emission and any isotropic radiation but also any continuous (unpulsed) emission from the pulsar itself or the Crab nebula. The two peaks and the background levels are indicated in Fig. 1a.

Added together, the light curves of Fig. 1a yield Fig. 1b which represents the best available database on the high-energy y-ray emission from the Crab pulsar and contains the first positive evidence in the energy range 50-3,000 MeV for highenergy y-ray emission in the 'interpulse region' between the two peaks of the PSR0531+21 light curve. Averaged over all the observations this amounts to $(15\pm4)\%$ of the total pulsed emission (that is the phase interval 0.10-0.62) in the energy range 50-3,000 MeV. This is not inconsistent with the upper limit of 15% derived earlier from the first observation alone¹³ and is about half of the corresponding contribution in the case of PSR0833-45 (ref. 14). Within the wide limits of error, there is no evidence for any variation with time in the relative flux in the interpulse region. An evaluation of the phase difference between the two peaks in Fig. 1b gives 13.0 ± 0.5 ms, in agreement with corresponding measurements at radio, optical and X-ray wavelengths^{15,16}. It is also possible to measure the peak widths which are found to be 1.6 ± 0.4 ms (FWHM) and $2.0\pm$ 0.5 ms (FWHM) respectively.

The light curves shown in Fig. 1 represent only a fraction of the photons from the pulsar, that is those with measured arrival directions, θ , (relative to the pulsar direction) within the selection cones. To obtain the best estimate of the total numbers of photons the distributions of their arrival directions were fitted with the point-spread function describing the instrument's angular resolution appropriate to the energy range and to the aspect angle, α , of the observation. This point-spread function is approximated by the expression¹⁷

$$h(\theta) = 2\pi\theta \exp(-(\theta/\theta_0)^{2c})$$

where c=0.8 for 50 < E < 150 MeV and 0.5 for 150 < E < 3,000 MeV. The value of θ_0 decreases with aspect angle in the lower energy range, from 4.8° at $\alpha=0^\circ$ to 4.3° at $\alpha=15^\circ$ and increases in the higher range, from 1.2° at $\alpha=0^\circ$ to 1.75° at $\alpha=15^\circ$.

Separate fits were made for each observation in each energy range and for the photons having phases within each of the two peaks. By integrating the fitted curves the total numbers of events contributing to each peak, P_1 and P_2 respectively, were determined. The ratio of the strengths of the two peaks was calculated and the results are presented in Table 1. These show that during the first observation the second peak was significantly stronger (relative to the first peak) than at later times. The effect is present in both energy ranges but is more pronounced below 150 MeV. Using a χ^2 analysis it has been found that the chance probability of the total effect is $\sim 5\%$.

The results presented here show no evidence for any change in the γ -ray light curve between 1976 and 1980. The departure from the mean lies in the earlier (1975) measurement. That result is consistent with the light curve measured by SAS 2 at energies above 35 MeV in 1972 and 1973¹⁸. It is estimated that, taking phase intervals as close as possible to those used in analysing the COS B data, the ratio of the second pulse to the first was 1.33 ± 0.39 at the time of the SAS 2 observation. This ratio is plotted together with those from COS B in Fig. 2. The probability that all these results represent chance fluctuations about a common mean is <1% combining a run test with the χ^2 analysis.

In a preliminary presentation of these results¹⁹ the γ -ray light curves for the Vela pulsar PSR0833-45 were also given for a similar time interval. No variation in the shape of the light curves was observed for that pulsar.

The measured variation in the Crab pulsar light curve cannot be ascribed to a change in the sensitivity of the instrument unless that change was energy dependent and the two pulses have markedly different energy spectra. Bennett et al.¹³ found no evidence for spectral differences sufficient to account for the effect seen here. Furthermore, a possible sensitivity change is not sufficiently energy dependent to influence the measured light curve of PSR0833-45 ¹⁹, for which the second pulse is known to have a harder spectrum than the first¹⁴.

The pulsar synchronizer on board COSB has allowed the long-term behaviour of the 2-12 keV X-ray light curve of the pulsar to be studied. Figure 3 shows the results for the three observations when the pulsar was within the field of view of the proportional counter. The shapes of these light curves exhibit excellent reproducibility. The same situation applies at

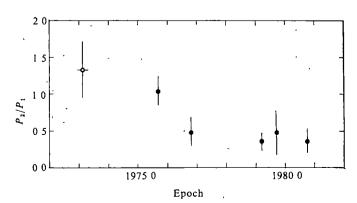


Fig. 2 Variation with time of the fraction of pulsed counts in the second pulse: ○, >35 MeV from SAS 2 data¹⁸; ●, 50-3,000 MeV from COS B data (present work).

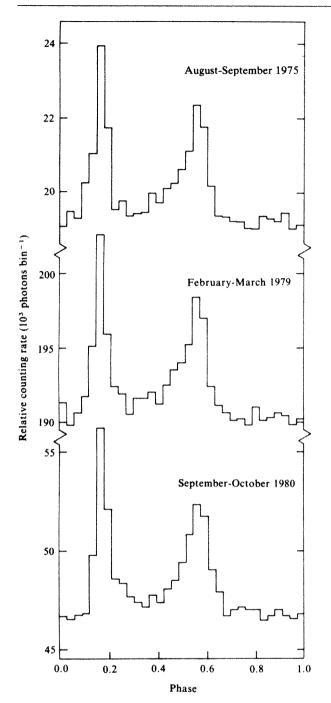


Fig. 3 X-ray (2-12 keV) light curves measured during observations 1, 39 and 54.

optical wavelengths where very small limits have been placed on the variation of the light curve²⁰. Above ~20 keV a decrease in the relative strength of the second X-ray peak between observations in 1969-70 ²¹⁻²³ and in 1974 ²⁴ has been claimed²⁵. However, the effect is probably due to differences in the definitions of the phase intervals of the two peaks by the original investigators. In low energy (1-20 MeV) γ rays light curves of the same shape were measured in October 1977 and May 1979 26 but over this limited time span there is no evidence in the COSB result for any variation. In the ultra-high energy range (>100 GeV) a variation in the strengths of different components of the light curve between 1971 and 1973 has been reported27

The observed variation in the high-energy light curve could be due to a change in the emission modes in one of the polar regions of the magnetosphere of the neutron star, but also to a change in the geometry, such as a decrease in the angle

between the magnetic and rotational axes. Whatever the explanation, the absence of the effect in the X-ray light curve points to different emission regions and/or production processes in the two energy ranges. The properties of the different components of the radio light curve support the presence of two beaming mechanisms ^{28,29}. Different emission regions or beaming geometries have also been proposed to account for differences between the radio, optical and y-ray light curves of PSR0833-45 (refs 28-30).

We thank Drs J. M. Rankin, V. Baroakoff and D. Ferguson for communicating radio data and Dr I. I. Shapiro for making available the MIT barycentric ephemeris.

Received 7 December 1981; accepted 24 February 1982.

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Was the 1970 geomagnetic jerk of internal or external origin?

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Annual mean data from worldwide magnetic observatories show that there was a jerk (step-change in the second time derivative) in the geomagnetic field, which took place over an interval of <2 yr around 1970. If such a short-lived phenomenon originated in the Earth's core, and were still detectable after passing through the mantle to the surface, this would imply a much lower conductivity for the lower mantle than had been believed previously. Here we show by an objective test that most of the jerk has an internal origin.

The geomagnetic field has a rich spectrum of temporal changes from audio frequencies to thousands of years. In general, the short-term variations (with time scales of a year or less) are of external origin relative to the Earth's surface, though they have a smaller internal component due to electromagnetic induction in the conducting layers of the Earth. The long-term variations, with time scales of several decades, are of internal origin. At intermediate time scales the picture is less clear; there are certainly external variations with a period of ~11 yr, associated with the sunspot cycle, and there are variations that occur over a few years which seem to be of internal origin. These short-term variations are of particular interest because, it they are real and originate from the Earth's core, they place constraints on the conductivity of the mantle through which they have to pass before being detected at the surface2

In 1967, using accepted values for the mantle conductivity, Currie³ deduced that no core signal with a period of ≤4 yr could penetrate the mantle and that all observed variations on a shorter time scale must be of external origin. Later, Alldredge4 raised this limit to 15 yr and suggested a correspondingly higher value of mantle conductivity. Other authors prefer lower conductivities and time scales.

The most convincing evidence for the lower values comes from the change in geomagnetic secular acceleration that occurred over an interval of 1 or 2 yr around 1970. This event was first reported by Courtillot et al.8 (although Mizuno had submitted an article nearly a year earlier describing similar effects for 1965 and 1974 restricted to the region of Japan) and has since been widely studied⁷⁻¹⁵. Although the effect is worldwide 14,15, it is most clearly seen in declination data from European observatories. See, for example, Fig. 1a, which shows plot of 3 yr running means of declination observed at Eskdalemuir, Scotland, and Fig. 1b, the first derivative of these data (secular variation) obtained by differencing successive means. The secular acceleration is the slope of curve b. It is reasonably constant before 1970 but changes suddenly at about that date to a new value, at which it remains for the rest of the interval shown.

The reality of this phenomenon, its worldwide distribution and the short interval over which it occurs are firmly established. However, although most authors 11,15 believe the effect to be of internal origin, their reasons for this are based on morphological arguments rather than objective measurement, and there are others 16,17 who believe that all such short-period variations are of external origin. We present here an objective separation into parts of internal and external origin and compare their sizes.

Gauss¹⁸ originally developed the method of spherical harmonic analysis to determine whether the main geomagnetic field was of internal or external origin, and the method may equally well be applied to time derivatives of the geomagnetic field. Here, we apply it to the change of secular acceleration that occurred around 1970. The data are annual mean values of the geomagnetic north, east and vertical components from the 83 observatories that operated throughout the interval 1961.0-1978.0 (ref. 19). For each observatory and element, means were formed for the centre of each even year by forming $(x_{n-1}+2x_n+x_{n+1})/4$, where x_n denotes the annual mean value for year n, giving eight bi-annual means, denoted (1)-(8). The pre-1970 secular acceleration was defined as [(1)-(2)-(3)+ (4)]/2 and the post-1970 secular acceleration as [(5)-(6)-

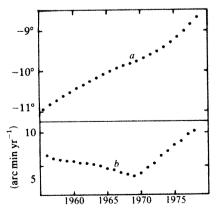


Fig. 1 Declination observed at Eskdalemuir, Scotland: a, mean values; b, secular variation obtained from first differences of the mean values. Note the sudden change of slope around 1970.

Table 1 Spherical harmonic coefficients of the change of secular acceleration that occurred around 1970

		Inte	rnal	Exte	
m	n	g_n^m $(nT yr^{-2})$	h_n^m (nT yr ⁻²)	g_n^m $(nT yr^{-2})$	$\binom{h_n^m}{n \operatorname{T} \operatorname{yr}^{-2}}$
0	1	-2.44 ± 0.27		-1.48 ± 0.43	
1	1		1.04 ± 0.29		
ō	2	1.03 ± 0.23		-0.78 ± 0.26	
1	2	0.59 ± 0.22	-2.40 ± 0.26		-0.29 ± 0.26
2	2	-0.68 ± 0.27	*****		-1.10 ± 0.29
õ	3	1.04 ± 0.16		*****	
ĭ	3	0.83 ± 0.18	-2.23 ± 0.20		0.94 ± 0.24
2	3	1.04 ± 0.20	-0.43 ± 0.20	_	-
3	3		Average or a		-0.88 ± 0.24
ő	4	*****			
i	4	0.28 ± 0.14		-0.28 ± 0.12	-0.20 ± 0.17
2	<u>A</u>	-0.77 ± 0.16	-0.41 ± 0.16	0.21 ± 0.15	0.35 ± 0.15
3	4	0.26 ± 0.16	0.95 ± 0.15	-0.35 ± 0.16	
4	4	-0.94 ± 0.23		0.73 ± 0.24	-0.74 ± 0.24

All coefficients that exceed their standard deviation are included, up to maximum degree and order 4.

(7)+(8)]/2. The jerk¹ is simply the difference between these quantities.

In the analysis, it is assumed that the jerk may be adequately represented by the spherical harmonic expansion of a potential field up to maximum degree and order 4 for both internal and external terms. The spherical harmonic coefficients are obtained by the method of least squares, solving for internal and external coefficients simultaneously, and evaluating their standard deviations in the usual way from the sum of squares of residuals, number of degrees of freedom and the leading diagonal of the inverse matrix. After some experiment with different sets of coefficients, the solution including all those that exceed their standard deviations and omitting all those that do not was found to be that given in Table 1. Following recommended geomagnetic practice²⁰, the coefficients are in the Schmidt quasinormalized form.

It is immediately obvious that most of the jerk is of internal origin: the mean square value21 of the internal part exceeds that of the external part by a factor of 3.4 ± 0.8 . Of the 10 largest coefficients, only 2 are for the external part. The larger of these, g_1^{0e} (using the notation of Chapman and Bartels² represents the field due to a ring of current above the Equator, which is precisely what would be expected for the external contribution. The only other external coefficient to exceed 1 nT yr^{-2} is $h_2^{2\epsilon}$; there is no obvious external source which would produce this term. The three coefficients that exceed 2 nT yr⁻² $(g_1^{0i}, h_2^{1i}, h_3^{1i})$ are all of internal origin. Part of the g_1^{0i} term could be due to induction by the current represented by g_1^{0e} , but, even if the whole Earth were a perfect conductor, this could not account for more than 0.74 nT of g_1^{0i} (ref. 23). We would not expect, and we do not find, any obvious pattern to the internal part, except the tendency15 for the coefficients to decrease in amplitude with increasing n. This tendency, which is more obvious when the coefficients are fully normalized, suggests that the jerk originates deep within the Earth rather than near the surface.

We conclude that internal sources can give rise to changes in secular variation on time scales as short as 1 or 2 yr and that these were indeed the major factor in the geomagnetic jerk that occurred around 1970.

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Received 19 January; accepted 5 March 1982.

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Intensity of the geomagnetic field near Loyang, China between 500 BC and AD 1900

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There are remarkably few data on the intensity of the geomagnetic field in early China. New results reported here indicate that the total intensity of the magnetic field of the Earth in the region near Loyang (lat. 34°42' N, long. 112°40' E) has changed considerably during the past 2,400 yr (500 BC-AD 1900). The maximum value reached about 1,700 yr ago is as much as 54% higher than the present one.

Loyang is the ancient capital of nine dynasties, so samples collected from the city and nearby regions are suitable for archaeomagnetic research. The samples used in the present work are mainly of three types: (1) baked earth from ancient pottery or brick kilns and ovens in residential structures; (2) grave bricks-some of them are engraved with accurate dates and place of their baking; (3) bricks from ancient structures such as buildings and city wall.

Table 1 gives the relevant data on samples collected from Loyang and Zhengzhou. The stability of the primary remanent magnetization was tested in all the samples and proved satisfactory1. The magnetization of the specimens was measured with an astatic magnetometer. The total intensity of the geomagnetic field was inferred from stepwise thermal demagnetization^{2,3} with temperature steps of 40-60 °C from 100 to ~600 °C. The slope of the natural remanent magnetization-partial thermal remanent magentization (NRM-PTRM) straight line was calculated using the method of least squares. The points in the

NRM-PTRM diagram which deviated systematically at low and high temperatures from a straight line were discarded.

The averaged data for each dynasty were obtained by using the method we have described earlier4, and are given in Table 1. Figure 1 shows variation of the total intensity of the geomagnetic field during the past 2,400 yr. The horizontal error bars represent the archaeological estimate of the duration for each epoch (or dynasty); the vertical error bars represent the standard deviation.

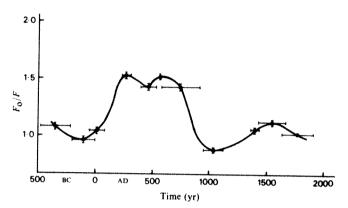


Fig. 1 The change in the ratio of the ancient intensity of the geomagnetic field, F_0 , to the present-day value F_0 in Loyang region.

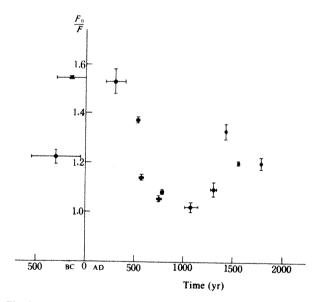


Fig. 2 Ratio of intensity of the ancient geomagnetic field (F_0) to that at present (F) in Japan³.

	Table 1 Data of samples from Zhengzhou and Loyang								
Sampling site	Lat. N	Long. E	Dynasty	Age	Sample type	N	F_0/F	F ₀ (Oe)	δF
Zhengzhou	34°54'	113°39′	Zhanguo	475-221 BC	Grave brick	2	• • • • • • • • • • • • • • • • • • • •		
Loyang	34°42′	112°40′	Western Han	206 BC-AD 8	Baked earth		1.09	0.60	0.01
Loyang	34°42'	112°40'	Han (Wang Mon)			4	0.96	0.53	0.01
Loyang	34°42′	112°40′	Wei-Jin	AD 9-23	Grave brick	. 5	1.05	0.58	0.01
Loyang	34°42′			AD 220-300	Brick, baked earth	2	1.54	0.85	0.01
		112°40′	Northern Wei	AD 386-534	Baked earth	3	1.43	0.79	0.02
Loyang	34°42′	112°40′	Northern Qi	AD 562-565	Grave brick	11	1.53	0.84	0.01
Loyang	34°42′	112°40′	Sui-Tang	AD 581-907	Brick, baked earth	10	1.43		
Loyang	34°42′	112°40′	Northern Song	AD 960-1127				0.79	0.02
Loyang	34°42′	112°40′	Early Ming		Brick, baked earth	8	0.88	0.48	0.01
Loyang	34°42′			AD 1368-1435	Brick	2	1.06	0.58	0.01
. , .		112°40′	Ming	AD 1436-1661	Brick	2	1.13	0.62	0.01
Loyang	34°42′	112°40′	Qing	AD 1631-1911	Brick	4	1.03	0.57	0.01

 F_0 , ancient intensity of the geomagnetic field; F, present-day intensity. N, number of samples.

3

It is clear from Fig. 1 that the total intensity of the geomagnetic field in the region near Loyang has changed. Between 400 BC and AD 1900 there are two minima, at about 100 BC and AD 1100, one (main) bimodal maximum between AD 250 and 600, and a smaller maximum at about AD 1550. The difference between the maximum (~85 µT) and both minimum values (~53 μT and ~48 μT respectively) amounts to about 37 µT. The length of time between the two minima is about 1,200 yr.

It is interesting to compare these data with those obtained from other locations in the world. For example, comparison of the results from Loyang with those given in ref. 5 indicates that they are broadly similar although the details vary. Moreover the variation of the ratio of the total intensity in early Japan to that in present-day Japan³ is in a good agreement with that for Loyang both in tendency and magnitude of changes during the past 2,400 yr, although there is a time delay over the whole interval (see Fig. 2). This is also the case in comparisons with data from Athens⁶, Czechoslovakia and central America⁷. It may be related to the westward drift of the non-dipole field.

We thank Drs Xu Jing-yuan and Huang Ming-lang for supplying samples used in work on the secular variation of the total intensity as well as on the direction of the ancient geomagnetic field, and Professor J. A. Jacobs for reading the manuscript and for valuable comments.

Received 21 October 1981, accepted 19 February 1982

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A spinel to β -phase transformation mechanism in (Mg,Fe)₂SiO₄

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Olivine of approximate composition Mg1.8Fe0.2SiO4 is a major constituent of the Earth's upper mantle. Structural changes, which occur in olivine as a function of increasing pressure and which involve the formation of the β -phase and spinel structure, γ -phase polymorphs, have therefore aroused considerable interest¹⁻⁴. The mechanisms of these transformations are of particular importance because of their effect on the properties of the mantle in the zone in which they occur. Several mechanisms have been proposed4-7, but remain controversial in the absence of sufficient observational data on intergrowths of reactant and product phases. Although these phases have been produced experimentally2, direct observations of such intergrowths have generally been made only on natural material from shock-produced veins in chondritic meteorites^{5,6,8}. Here we report observations made on the natural, high-density polymorphs of (Mg,Fe)₂SiO₄ found in the Peace River meteorite, which reveal that the mechanism for the spinel to β -phase transformation involves the topotactic replacement of the spinel polymorph by single, β -phase grains.

We have previously described^{5,6} the microstructures in the spinel polymorph of (Mg,Fe)₂SiO₄ (ringwoodite) from the Tenham chondritic meteorite, and suggested that the defects (Fig. 1 in ref. 5) found in the spinel were due to the partial inversion of ringwoodite to the β -phase. The defects found in the spinel lie on $\{110\}$ planes, and contrast analysis indicates^{6,8} that for a (110) defect, the displacement vector is of the type a/4[110], or equivalently $a/4[1\overline{1}2]$ (refs 8.9). The defects are therefore stacking faults, which may be viewed either as Frank-type faults corresponding to the removal of a stoichiometric (Mg,Fe)2SiO4 layer accompanied by an a/4[110] displacement, or as Shockley-type faults corresponding to an $a/4[1\overline{1}2]$ shear. Stacking faults of this type leave the oxygen sub-lattice unaffected, but change the cation distribution so that in the fault plane the structure is that of the β -phase. The spinel and β -phase structures are closely related 10,11, and indeed the spinel structure can be completely transformed into that of the β phase by the introduction of two a/4[110] (110) stacking faults per unit cell. The transformation of spinel to β -phase may, therefore, be considered relatively simple, and one which would, in suitable conditions, be favoured kinetically over the reconstructive inversion to olivine. In this respect, our conclusions differ from those of Madon and Poirier⁸ who assume that the faults in ringwoodite result from the impingement of spinel domains nucleated at various places within the olivine crystal during the prograde transformation, rather than being associated with a post-shock, retrograde process. Here, we present further evidence to support our suggestion that the development of stacking faults in the spinel phase is indeed related to the mechanism of the spinel to β -phase transformation.

The Peace River meteorite¹² (classified as an L6 olivinehypersthene chondrite) contains shock-produced veins, which pervade the body of the meteorite in much the same way as the veins found in other shocked chondrites (for example, Tenham, Coorara, Catherwood and Coolamon)¹³⁻¹⁶. In fragments found within these veins, the constituent olivines and hypersthenes have been transformed to their high-density polymorphs, ringwoodite and the garnet-structure majorite respectively. In general, these 'high-pressure phases' have remained, quenched-in after an extraterrestrially produced shock event. The vein studied in the Peace River meteorite differs significantly, however, from those described in other meteorites, in that it contains significant quantities of the β -phase polymorph of $(Mg, Fe)_2SiO_4$. The β -phase occurs as microcrystalline aggregates which pseudomorph previously existing olivine fragments within the vein. The fragments rarely exceed 0.5 mm in diameter. Electron microprobe analyses of β -phase aggregates yield an average, ideal formula for the observed β -phase of (Mg_{1.4}Fe_{0.5}Mn_{0.1})SiO₄. Representative analyses of a β-phase aggregate and an untransformed olivine are given in Table 1; within experimental limits, no compositional differences between the (Mg,Fe)₂SiO₄ polymorphs could be detected. Least-squares refinement of X-ray powder data obtained from separated β -phase fragments yielded orthorhombic cell parameters of a = 5.70(2), b = 11.51(7) and c = 8.24(4). The calculated cell volume for the observed β -phase is 541(3)Å, and with z = 8, the calculated density on the basis of the idealized formula is 3.84 g cm⁻³.

Transmission electron microscopy (TEM) shows that relatively large fragments within the shock-vein are composed of granular aggregates of well formed, micrometre-size β -phase grains, with intergranular boundaries usually meeting at triple points (often with interfacial angles close to 120°). Grains of β -phase are also found replacing grains of ringwoodite (Fig. 1). The spinel grains invariably carry a high density of a/4110 stacking faults (identical to those found in the Tenham meteorite^{5,6}). There is an occasional slight (±2°) misalignment of the β -phase lattice relative to the spinel lattice, but electron diffraction confirms that the phases are essentially orientated such that $[010]_{\theta} | [110]_{\gamma}$ and $[001]_{\theta} | [001]_{\gamma}$. The phases share, therefore a continuous, near cubic-close-packed oxygen sublattice.

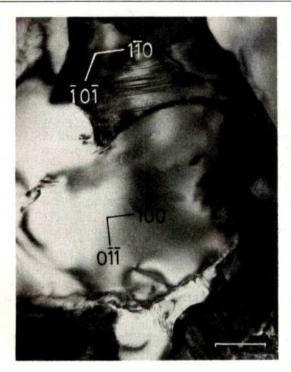


Fig. 1 Electron micrograph of a fault-free β -phase grain, topotactically replacing faulted ringwoodite. The two phases are related such that $[010]_{\beta} ||[110]_{\gamma}$ and $[001]_{\beta} ||[001]_{\gamma}$. A subgrain boundary separates the two phases, but other examples were found which had coherent interfaces. Scale bar, 0.2 µm.

The textures observed are interpreted as resulting from postshock inversion of the spinel polymorph to a lower density phase, in response to a reduction in pressure. The proposed mechanism for the observed transformation of spinel to the β-phase involves two stages. The process initially is a cooperative or martensitic one, and probably proceeds by the propagation of low-energy¹⁷, Shockley partial dislocations of the type 1/4[112] (110) through the spinel, to produce the observed stacking faults on {110} planes. These faults form on all {110} sets of planes in the spinel, however, and prevent the simple cooperative formation of a single, β -phase grain from the host spinel (a process which would require faults on only one set of (110) planes). As the density of faults increases, a critical volume of β -phase is produced locally, which then grows and topotactically replaces the host. As the β -phase region grows, the faults in the spinel are swept before the advancing interface (Fig. 1) to produce a relatively fault-free grain of β -phase. Occasionally, relict faults are found in the β -phase grains (Fig. 2), which high resolution electron microscopy has shown to be of the type 1/4[010] (010) (expressed in terms of the β -phase lattice), and which are therefore complementary to those found in

Table 1 Electron microprobe analyses of β -phase aggregates and shocked olivine crystals form the Peace River meteorite

	β-phase (wt%)	Olivine (wt%)
SiO ₂	38.37	38.16
Al ₂ O ₃	0.00	0.00
Cr ₂ O ₃	0.00	0.00
MgO	38.31	38.38
CaO	0.05	0.00
MnO	0.31	0.40
FeO	23.10	23.57
CoO	0.20	0.00
NiO	0.11	0.05
Total	100.45	100.56

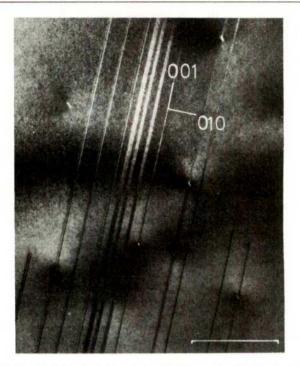


Fig. 2 Residual faults found in a β -phase grain. The faults are of the type 1/4[010] (010), and gives rise to the spinel cation distribution within the fault plane. Faults are terminated or displaced at dislocations. Scale bar, 0.1 µm.

spinel, that is locally retaining the spinel cation distribution within the β -phase matrix. The faults are often complex in detail and are frequently terminated at dislocations within the grains. They are interpreted as being residual features preserved as a result of the relatively rapid, post-shock growth process, and are the subject of continuing research.

The spinel to β -phase inversion is expected to occur^{7,18} within the mantle, at a depth close to 400 km, as a result of the peritectoidal relationship between the olivine, spinel and β phase polymorphs of magnesium-rich members of the (Mg,Fe)2SiO4 system. However, considering the different thermal regimes involved, it is difficult to assess whether the transformation of spinel to β -phase occurs in the mantle by the same mechanism as the one which has been inferred from the textures in the Peace River meteorite. A mechanism of this type occurring in the mantle would have as the rate-controlling step the diffusion of atoms across the β -phase/spinel interface. The initial cooperative or martensitic stage of the mechanism would, however, particularly weaken the spinel with respect to shearing stresses on {110}. Suitable shears may allow the transformation to proceed to completion by a single, military process, and in this case, the rate-controlling step would be the formation and glide of partial dislocations through the spinel lattice. On the basis of these suggested mechanisms, it may be inferred, therefore, that the overall kinetics of the spinel to β -phase phase transformation will be particularly sensitive to shear. In addition, the resistance of spinel bearing mantle to shear stresses would be significantly reduced during the transformation to the β-phase, and would affect mantle rheology.

We acknowledge the help and advice of Dr S. O. Agrell, and the NERC for electron microscope facilities. G.P.D. also acknowledges the receipt of Research Fellowships from the NERC and from Clare College, Cambridge.

Received 22 December 1981; accepted 26 February 1982.

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Winchite and the actinolite-glaucophane miscibility gap

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Leake et al.1 have recently emphasized the apparent scarcity of winchite s.l., NaCa(Mg, Fe²⁺)₄(Al, Fe³⁺) [Si₈O₂₂/(OH)₂] (ref. 2), an amphibole intermediate in composition between the glaucophane-riebeckite series of high-pressure blueschists and the tremolite-actinolite series of lower-pressure greenschists. The rarity of winchite is considered consistent with the existence of a miscibility gap^{3,4} between these two amphibole series at the metamorphic P-T conditions normally attained in nature. Here we present optical and X-ray properties of Mg- and Al-rich winchite from Venezuela and draw attention to several other recent winchite analyses. In fact, as Schliestedt's new data on co-existing sodic and calcic amphiboles in blueschists show, only the present Mg-Al-rich winchite definitely lies within the above miscibility gap.

Winchite was identified in an amphibole-eclogite found in talus near El Valle del Espiritu Santo, Margarita Island, Venezuela (63°53'10" W, 10°58'55" N; Venezuela grid ref. no. 40322142). Field relationships of similar occurrences on Margarita indicate that the eclogite is a metabasalt pod in quartzmica schist of epidote-amphibolite facies grade. The bulk-rock chemistry of the eclogite indicates a tholeiite of MORB affinity6. Winchite is found with omphacite, garnet, rutile and quartz, as well as with minor epidote, albite, opaque oxide, sphene and apatite. Thin actinolitic rims overgrow some of the winchite grains.

Electron microprobe analyses (Table 1) indicate that the amphiboles are winchites grading to alumino-winchites (Al> 1.0)2. Figure 1 shows that the departure from ideal end-member winchite composition is due mainly to a minor amount of edenite substitution, (Na, K) + Al - Si, giving A-site occupancy totals of 0.02-0.18 atoms per half-unit-cell formula, and more $(Al, Fe^{3+}) + Al \rightarrow$ substitution, Tschermak's extensive (Mg, Fe²⁺)+Si, leading to Si totals of 7.56-7.78. Figure 1 also gives published data of samples that classify as winchite, although the samples are markedly more iron-rich and would be classified as ferro- or ferri-winchite2. All analyses are from blueschist terranes. Clearly, winchites approaching the ideal end-member are not uncommon in nature.

We have determined the optical and X-ray properties of the Venezuelan winchite (Table 2) on a single cleavage fragment $\sim 50 \times 50 \times 200$ µm in size. The optical properties were corroborated on several other fragments and are considered to be representative. Indices of refraction and 2V were measured using the microrefractometer spindle stage of Medenbach⁷, while X-ray data were collected on a four-circle automatic diffractometer. A scan of 278 reflections between 0 and 35° MoK_{α} revealed no reflections violating monoclinic C symmetry.

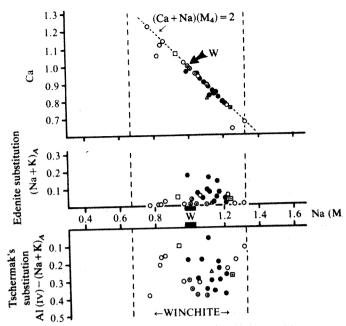


Fig. 1 Plot of $Na(M_4)$ against edenite substitution, $(Na+K)_A$, Tschermak's substitution, Al (IV)-(Na+K)A, and Ca all referred to half-unit-cell formula. Data sources: •, present study; O, ref. 5; □, ref. 14; △, ref. 8; ○, ref. 15; □, ref. 9. Fe³⁺/Fe² for all analyses are calculated as for Table 1. W, ideal end-member winchite.

Table 1 Representative microprobe analyses of Venezuelan winchite

Ana	lysis 1.1 (alumino-	winchite)	Ar	ialysis 8.2	(winchite	e)
Ana SiO ₂ TiO ₂ Al ₂ O ₃ Fe ₂ O ₃ FeO MnO MgO CaO Na ₂ O K ₂ O Total	55.00 	Si Al Total Al Ti Fe ³⁺ Fe ²⁺ Mn Mg Total Ca Na K	7.70 0.30 8.00 1.04 - 0.37 0.83 0.01 2.75 5.00 0.83 1.21 0.02 2.06	SiO ₂ TiO ₂ Al ₂ O ₃ Fe ₂ O ₃ FeO MnO MgO CaO Na ₂ O K ₂ O Total	54.41 0.20 7.00 4.72 6.09 0.04 14.25 6.32 4.01 0.16 97.20	Si Al Total Al Ti Fe ³⁺ Fe ²⁺ Mn Mg Total Ca Na K	7.63 0.37 8.00 0.79 0.02 0.50 0.71 0.01 2.97 5.00 0.95 1.09 0.03 2.07

The Fe3+/Fe2+ ratio was calculated by normalizing tetrahedral and octahedral occupants to 13 at constant 23 oxygens. This procedure is acceptable for sodic-calcic amphiboles

There is no evidence of exsolution. Table 2 shows that the optical and unit cell properties of Venezuelan winchite lie between those of tremolite and glaucophane, as expected.

The occurrence of winchite amphibole has been interpreted by Brown⁸ and Green and Spiller⁹ to indicate closure of the tremolite/actinolite-glaucophane/riebeckite miscibility gap. Recent data from Schliestedt⁵ on coexisting amphibole pairs from Sifnos, Greece, indicate, however, that the multidimensional nature of the miscibility gap must be considered. Two projections of the latter, based on Schliestedt's data, are given in Fig. 2. Although the exact shape of the miscibility gap in $Na/Na + Ca - Fe^{2+}/Fe^{2+} + Mg - Fe^{3+}/Fe^{3+} + Al$ space is unknown, simple geometrical reasoning suggests that only the Venezuelan winchite appears to lie in the gap with any certainty. Thus only magnesian, alumino-winchites indicate P-T conditions exceeding the critical temperature of the gap. Iron-rich

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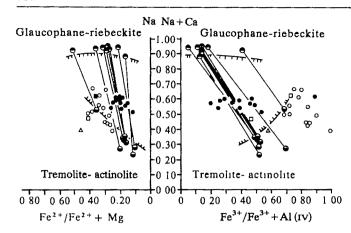


Fig. 2 Location of actinolite/tremolite-glaucophane/riebeckite miscibility gap in blueschists after data on coexisting amphiboles from Schliestedt⁵ (🔿) compared with winchite compositions from Venezuela and the literature. The hatched boundary represents approximate minimum solvus location. Other symbols as for Fig. 1.

Table 2 Optical properties and lattice parameters of Venezuelan winchite compared with tremolite 11,12 and glaucophane 13 chosen to reflect similar Fe²⁺/Mg and Fe³⁺/Al ratios

	Tremolite	Winchite	Glaucophane
а	9.818 (5)	9.71(1)	9.595 (2)
b	18.047 (8)	17.93 (2)	17.798 (3)
c	5.275 (3)	5.283 (6)	5.307(1)
β	104.65 (5)	104.3 (1)	103.66(1)
V	904.2 (6)	891 (2)	880.6
n_x	1.622	1.6294 (5)	1.626
	Pale yellow-green	Colourless	Nearly colourless
n_{y}	1.633	1.6428 (5)	1.644
•	Pale green	Light blue-violet	Light purple
n_z	1.644	1.6496 (5)	1.646
	Pale blue-green	Light blue	Light blue
Δ	0.022	0.020	0.020
$2V_x$	80-85°	$64 \pm 2^{\circ}$	38.1°
Zc	14°	16°	6°

winchite can be stable in normal blueschist-facies conditions where magnesian, alumino-winchite is not. Thus the latter should not be found in typical blueschists.

The upper temperature stability limit for winchite is unknown. For most common metabasaltic compositions metamorphosed in intermediate- to high-pressure conditions, however, increasing Tschermak's substitution with increasing temperature in the amphibole of the basic assemblage will lead to more Si-deficient amphibole compositions such as barroisite or hornblende. Thus the common range of P-T stability of magnesian, alumino-winchite must be severely restricted. For Venezuelan, winchite-bearing amphibole Rudolph⁶ has estimated equilibration conditions of at least 10 kbar and 600 ± 30 °C for the pyroxene-garnet pair, but it is not clear whether winchite growth was approximately coeval or occurred significantly later in the metamorphic cycle.

We thank W. Gebert for supplying the X-ray data, and W. Schrever and R. Grapes for helpful discussions. The research was partly supported by grant Ma 689/1 of the Deutsche Forschungsgemeinschaft, Bonn, to W.V.M.

Received 4 January; accepted 5 March 1982

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History of the continental lithosphere recorded by ultramafic xenoliths

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Since the development of the plate tectonic theory, the formation, structure and properties of the continental lithosphere have been widely debated1-3. By analogy with petrological models for the oceanic lithosphere generated at ridges4-6, the continental lithosphere has been interpreted in terms of a comprehensive mechanical and petrogenetic unit by considering, for example, that the continental crust was differentiated through a process such as zone melting, the lithospheric mantle being the residue of this extraction process^{7,8}. However, geological observations show that formation of the continental crust was quite complex, including sedimentation, metamorphism and orogenesis, and not simply direct magmatic segregation. Underneath its crust, the continental lithosphere, mainly comprises peridotites and in this respect, it resembles the oceanic lithosphere. This similarity, which is corroborated by seismic data, does not, however, mean that a chemical coupling exists between the subcontinental lithospheric mantle and continental crust in a similar way as for the oceanic lithosphere. Although magma generation and eruption through the continental lithosphere contribute to the growth of the continental crust and to the depletion of the subcontinental lithospheric mantle in incompatible elements, no simple chemical coupling should exist if the continental lithosphere were created by thermal accretion. The petrology and geochemistry of the continental lithosphere can also be studied using xenoliths brought up to the surface by kimberlitic or alkalic volcanics. This technique attracted much attention because of the determination of pyroxene geotherms9 and the claim that it permits the description of the palaeogeothermal structure of continents and oceans 10,11. Although some of the original conclusions seem premature12 the basic idea is sound. We have now used radiogenic tracers, the ⁸⁷Rb-⁸⁷Sr system, to study the creation of the continental lithosphere. We present here results on garnet lherzolite xenoliths from southern African kimberlites and discuss their geochemical and geodynamical implications.

The major and trace element chemistry of the constituent minerals of most of the samples studied here (see Table 1) has been described elsewhere¹³. The olivine-bearing xenoliths in kimberlites can be ascribed to two textural groups: granular peridotites and sheared lherzolites. Whereas the sheared samples have a 'pristine-mantle-type' chemical composition, the granular ones show a very complex chemistry, depleted in the basaltic components while enriched in some trace elements. Clear evidence for mantle metasomatism has also been reported¹³ for some granular samples.

To study the isotopic characteristics of xenoliths, the minerals enriched in the elements of interest and which are insensitive to secondary reaction processes must be separated. Thus, we separated diopside and garnet as they are the main carriers of

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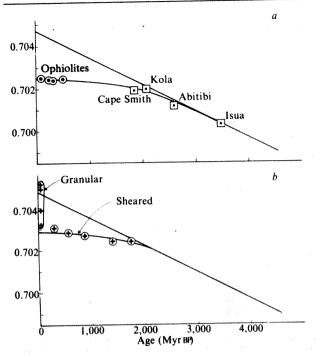


Fig. 1 a, ⁸⁷Rb-⁸⁷Sr diagram showing the thermal evolution of a garnet peridotite under rehomogenization; b, ⁸⁷Rb-⁸⁷Sr diagram for sheared xenoliths from kimberlites showing the localization of several garnet-diopside isotopic tie-lines.

Sr and Rb in these rocks. Separation was done by hand-picking, the purity was checked under a microscope and the separates were washed in high-purity acetone and water to eliminate any surface contamination. The Sr isotopes were analysed using the technique described in ref. 14; the blanks and the results on the standards are given in Table 2. Rb and Sr concentrations were obtained by an isotope dilution technique.

The analytical results summarized in Table 3 show that diopsides have very low Rb/Sr ratios and thus tend to fossilize the ⁸⁷Sr/⁸⁶Sr initial ratio of a mineral assemblage. The observed variation in strontium isotopic composition among diopsides (0.70245–0.70512) clearly demonstrates the heterogeneity of the initial ratio for the continental lithospheric mantle at the time of the kimberlite eruptions.

Such an heterogeneity may reflect either an intrinsic isotopic heterogeneity of the mantle rocks if the assemblages are in internal equilibrium, or different episodes of formation of the continental lithosphere corresponding to the different initial ratios. In an attempt to answer this question we also analysed the garnets, which are the second largest reservoir of Sr after diopside.

The most remarkable result is that diopside and garnet in a given granular sample have very similar ⁸⁷Sr/⁸⁶Sr ratios, whereas the sheared lherzolites yield very different Sr isotope

Table 1 List of samples Chemical type Textural type Locality No. Porphyroclastic Premier Mine W 397 Thaba Putsoa Fluidal mosaic PHN 1611 Thaba Putsoa Mosaic PHN 1597 Thaba Putsoa Mosaic PHN 1566 Thaba Putsoa Porphyroclastic THA 3 Ш Coarse granular PHN 1569 Thaba Putsoa II Coarse granular Bultfontein AJE 25 \mathbf{II} Matsoku Coarse granular MAT 7 Coarse granular (Spinel Matsoku **MAT 10** lherzolite)

ratios for these minerals. In other words, considering the ⁸⁷Sr growth since the time of kimberlite eruption, diopside and garnet are close to internal isotopic equilibrium for the granular xenoliths while they are in isotopic disequilibrium for the sheared samples. This behaviour is most surprising in the light of the geothermometry data, as the granular xenoliths typically yield a temperature around 1,000 °C compared with 1,300 °C for the sheared samples. How can rocks with large crystals such as the granular samples be isotopically homogenized at 1,000 °C whereas higher temperature facies with a finer grain-size are in isotopic disequilibrium?

If the rate of re-equilibrium (presumably controlled by the diffusion rate of elements in minerals) were much greater for the major elements than for Rb and Sr, the Sr isotopic disequilibrium could be preserved while the major elements would be completely re-equilibrated. Diffusion of major and trace elements in silicate minerals is still poorly understood. Recent

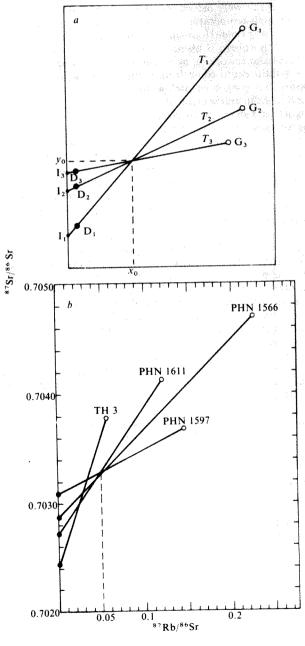


Fig. 2 a, $(^{87}\text{Sr}/^{86}\text{Sr}$, time) isotopic evolution diagram for the sub-oceanic mantle, after ref. 19; b, (apparent age, $^{87}\text{Sr}/^{86}\text{Sr}$ initial ratio) diagram for peridotite xenoliths; sheared nodules Thaba Putsoa.

work¹⁵ on Ca self-diffusion suggests a diffusion coefficient varying from $7.0\times10^{-12}\,\mathrm{cm^2\,s^{-1}}$ to $2.2\times10^{-11}\,\mathrm{cm^2\,s^{-1}}$ between 1,300 and 1,200 °C, respectively, with an upper limit of $2\times10^{-12}\,\mathrm{cm^2\,s^{-1}}$. For Fe-Mg interdiffusion in Ca-rich pyroxene, only Sanford and Huebner's¹6 calculated minimum value of $4\times10\,\mathrm{cm^2\,s^{-1}}$ at 1,050 °C is available. Sneeringer and Hart¹7 obtained a diffusion coefficient for Sr in diopside of $2.7\times10^{-12}\,\mathrm{cm^2\,s^{-1}}$ in the range 1,250-1,300 °C. None of these values is very well established, but they seem to suggest that there are no significant differences between the rate of re-equilibration of major elements and Sr. One may thus speculate that, for a given mineral couple, Sr isotopes are equilibrated only if the major elements have also reached equilibrium.

Therefore, we conclude that pyroxene geotherms or similar features have no simple interpretation. More probably, the (P, T) determination corresponds to fossil conditions, but different rocks have recorded these conditions at different times.

If the minerals in the sheared nodules form an internal isochron of age T_1 relative to a parent-daughter system, the P-T conditions calculated for the minerals correspond to the time T_1 . If this were indeed the case, it would call for a process in which high-temperature equilibrium was frozen and preserved for a long time. Hofmann and Hart¹⁸ stated that "subcontinental lithosphere is essentially protected against high temperatures and reworking by virtue of the overlying crust" and the xenoliths could come from mantle levels where the temperature has long been below the closure temperature for diffusion of the major elements as well as Sr.

Figure 1a is an isochron diagram showing the diopside-garnet joins for sheared xenoliths from the Thaba-Putsoa pipe. Note that these tie lines intersect each other almost at a unique point. Furthermore, the steeper the slope of the tie-lines, the lower the 87Sr/86Sr ratios for the diopside. We consider this as a strong evidence against impurity effects, particularly on garnet separates, as we expect that random impurity effects would yield random intersections of the tie lines. Thus, we would closely approximate the ideal situation depicted in Fig. 1b in which the system is re-equilibrated isochemically at different times $(T_1, T_2, \text{ and so on})$. The fact that diopside and garnet practically account for the total content in Rb and Sr of the garnet lherzolites supports our hypothesis. An important geochemical implication is that the sheared xenoliths from Thaba Putsoa represent various portions of a mantle system with a common Rb/Sr ratio, the value of which is quite low. The fact that the sheared-lherzolite mineral phase (and thus the whole rocks) show a remarkable coherence relative to some trace elements¹ is consistent with our speculation.

The situation illustrated in Fig. 1b may be readily converted into another linear relationship by plotting slopes against intercepts. Because Fig. 1 is an isochron diagram, the slopes rep-

Table 2 Isotopic analysis results Rienks Sr Rb Date (ng) (pg) (pg) 26 April 1978 0.46 26 58 92 22 May 1978 59 0.839 June 1978 13 45 37 16 October 1978 43 117 Standards 87 Sr/ 86 Sr $\pm \frac{2\sigma}{\sqrt{n}}$ 12 March 1978 0.71016 ± 0.00006 3 April 1978 0.71018 ± 0.00007 14 April 1978 0.71015 ± 0.00006 16 May 1978 0.71016 ± 0.00006 19 July 1978 0.71010 ± 0.00009 16 October 1978 0.71007 ± 0.00005 23 October 1978 0.71012 ± 0.00004

Table 3 Analytical results for kimberlite nodules

	⁸⁷ Sr/ ⁸⁶ Sr	⁸⁷ Rb/ ⁸⁶ Sr	Rb (p.p.m.)	Sr (p.p.m.)
Granular nodi	iles			
MAT 7				
Diopside	0.70323 ± 10	0.00087	0.081	270
Enstatite	0.70380 ± 12	0.381	1.40	10.64
Garnet	0.70408 ± 16	1.53	1.10	2.08
MAT 10				
Diopside	0.70496 ± 10	0.00042	0.013	91.5
Olivine	0.70799 ± 10	0.00058	0.014	6.95
PHN 1569				
Diopside	0.70398 ± 6	0.00017	0.031	514
Enstatite	0.70379 ± 12	0.0363	0.104	8.33
AJE 25				
Diopside	0.70512 ± 10	0.00044	0.032	209
Enstatite	0.70494 ± 7	0.234	0 440	5.50
Garnet	0.7056 ± 2	0.252	0.105	1.21
Sheared nodu	les			
ГНА 3				
Diopside	0.70243 ± 6	0.00056	0.0013	83.3
Enstatite	0.7051 ± 4	0.0103	0.020	5.61
Garnet	0.70379 ± 15	0.056	0.0427	2.20
W397				
Diopside	0.70245 ± 7	0.00097	0.034	98.3
Garnet	0.7253 ± 2	1.10	0.844	2.21
RT	0.71772 ± 9	0.784	2.79	10.3
PHN 1611				
Diopside	0.70272 ± 4	0.0018	0.074	116
Enstatite	0.70397 ± 10	0.460	0.946	5.94
Garnet	0.70413 ± 15	0.115	0.426	10.7
RT	0.7035 ± 1	0.562	7.82	40.3
PHN 1566				
Diopside	0.70285 ± 6	0.00034	0.011	94.3
Garnet	0.7047 ± 3	0.224	0.182	2.35
Enstatite	0.70489 ± 14	0.113	0.134	3.44
PHN 1597				
Diopside	0.70309 ± 9	0.0012	0.035	82.6
Garnet	0.70367 ± 6	0.143	0.076	1.54
Olivine	0.70383 ± 15	0.379	0.611	4.48

resent times and the intercepts, the initial ⁸⁷Sr/⁸⁶Sr ratios and hence the Sr isotopic characteristics of this particular mantle system can be further examined in terms of its Sr isotopic evolution. Figure 2 shows a good linear fit, not only for the four sheared xenoliths from Thaba Putsoa but also for a sample from the Premier Mine pipe (1,400-Myr old kimberlite).

Compared with the bulk earth Sr isotope evolution curve, the sheared xenoliths define a Sr isotopic evolution for a depleted mantle; this evolution trend is very similar to that for the oceanic lithosphere¹⁹. If we calculate the $^{87}\text{Rb}/^{86}\text{Sr}$ ratio for the uncontaminated whole rocks, through the isotopic evolution diagram (Fig. 1b), we get values around 0.05, which undoubtedly indicates a depletion since the bulk earth value is 0.09.

The Sr isotopic characteristics summarized above may be interpreted in terms of continuous or episodic thermal accretion of the continental lithosphere at its base. When an old shield continuously accretes this lithosphere the earliest fraction producing the uppermost lithospheric mantle is derived from older mantle which, until that time, has been undepleted; the youngest fraction producing the lower part of the continental lithosphere shows the reverse trend.

In contrast, we can admit that continental lithosphere was mostly created just after the local orogenic processes which built the local continental crust. The subsequent cooling of such episodic accreting lithosphere is not uniform with depth. Therefore, the isotopic freezing occurs at different times for different depths, creating the observed isotopic zonation.

In either model, we must consider independently the granular xenolith data. These samples are depleted in major elements but some are quite undepleted in terms of their ⁸⁷Sr/⁸⁶Sr ratios. The characteristics of these xenoliths may be explained through a two-stage model. At their depth level, melting occurred,

creating some continental basalts such as the Karroo basalts. The granular facies would thus represent residues after basalt extraction events which are indeed quite recent. However, some of the granular samples correspond to higher level and they have a ⁸⁷Sr/⁸⁶Sr signature quite undepleted, others correspond to a lower level and are more depleted. Such volcanism extracted basalts, leaving residual material such as the granular-facies rocks, yield fluids which induced mantle metasomatism. The combination of the two phenomena created the complexities in the chemistry and isotopic characteristics of the continental lithosphere.

We thank J. C. Mercier, S. R. Hart, and B. Dupré for helpful discussions. This is I.P.G.P. contribution NS/571.

Received 12 October 1981, accepted 12 February 1982.

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Origin of longitudinal triangular ripples on the Nova Scotian continental rise

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Since the recognition of current-produced bedforms in the deep ocean, attempts have been made to predict direction, intensity and variability of abyssal flow from the nature of sea floor irregularities (see refs 1-3). Longitudinal triangular ripples (LTRs) are intermediate-scale bedforms (<20 cm high, metres long) that are becoming recognized as important and geographically widespread predictors of bottom-current flow. I describe here recent sea floor photographs of LTR fields on the lower continental rise off Nova Scotia which show that the bedforms develop in fine-grained, cohesive sediment with an alignment parallel to the regional contours and to mean current direction. An observational model for LTR formation is proposed, wherein LTRs are deposited initially as 'tails' behind large sea floor biological mounds and are subsequently constructed and propagate in the direction of mean current by sediment transport oblique to the bedform axis. This transport is induced by short-term currents that deviate from mean current direction and cause formation of a separation bubble on one side or the other of the LTR crest.

Current-produced bedforms in the deep ocean range from mere lineations a few millimetres high to abyssal sediment waves up to 100 m high and several kilometres in wavelength. Among the larger bedforms that can be photographed with conventional deep-sea cameras are LTRs ranging up to ~15 cm high, 1 m wide and several metres in length. Such features appear to align with the mean current direction, and they have been photographed at depths of 4,600-5,400 m along the lower continental rise off eastern North America⁵⁻⁷, at the foot of the Bahama Banks⁸, on the north-east Bermuda Rise⁹, and in the

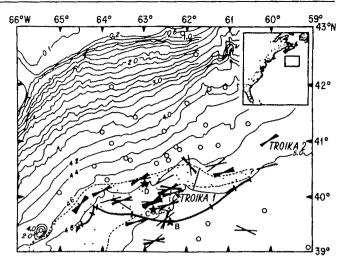


Fig. 1 Location and orientation of longitudinal triangular ripples¹⁶. Note that even NW-SE oriented LTRs at right centre parallel local bathymetric contours. Troika-1 was a 3.4-km photographic transect and Troika-2 an 8.1-km transect. O, Photograph stations with no LTRs. Surface sediment in stippled area contains more than 10% sand. Bathymetry in corrected metres. *, Locations of current-meter records ¹⁴: B, 15-day record; C, 215-day; D, 230-day; progressive vector diagram at B is tick-marked at 2-day intervals.

south-west Indian Ocean¹⁰. Recent detailed sea floor investigations for the high energy benthic boundary layer experiments (HEBBLE)¹¹ have shown that LTRs are widespread at depths of 4,700-5,100 m on the lower continental rise off Nova Scotia (Fig. 1). The bedforms have been photographed with both a Ewing-Thorndike bottom-bounce camera 12 (high-angle oblique view) and a Troika camera sled¹³ (low-angle oblique view).

The LTRs on the Nova Scotian continental rise are best developed at depths of 4,800-5,000 m. The surface sediment is a sandy silty clay (<20% sand, 30-50% silt and 30-60% clay). LTRs tend to be better developed and more frequent on the southwestern part of the continental rise where sediments also have higher sand content (Fig. 1). They generally align within 10°-15° of the strike of the bathymetric contours. Individual LTRs are up to 15 cm high, about 1-5 m long, and their spacing ranges from <1 m to several tens of metres (Fig. 2). Width ranges up to ~1 m and varies roughly in proportion to height. The ripples are mostly bilaterally symmetrical, and they are occasionally slightly sinuous. Ripple-crest height is not constant but varies by a few centimetres along the length of a ripple (Fig. 2e). Where the tails (downstream, south-west ends) of the LTRs are observed, they blend smoothly into the sea floor. In contrast, the heads of the LTRs merge smoothly with the sea floor in only about one-third of the ripples. The remaining ripples have a distinct bulge at the head, often recognized as a biologically produced mound (Fig. 2b, d). Thus it seems that the LTRs commonly, if not ubiquitously, have formed behind such obstacles to flow.

Associated with the LTRs are various other current-produced bedforms. Most common are fine lineations a few millimetres high, and crag-and-tail features up to 1 cm high and several centimetres long; these often occur on the LTRs (Fig. 2). Orientation of these smaller features is more irregular than that of larger bedforms, suggesting that there is significant shortterm (days to weeks?) variability in the direction of currents that produce the small-scale bedforms. Measured currents in this area do show such variability superimposed on a mean contour-parallel flow towards the south-west¹⁴. The LTRs themselves appear to be 'vector-averaging current vanes' oriented in the direction of long-term mean flow.

The small-scale bedform patterns superimposed on the LTRs are pertinent to LTR origin. Close study of photographs shows three kinds of ripple-crest development. Least common is a sharp, symmetrical crest: more often the crest is rounded

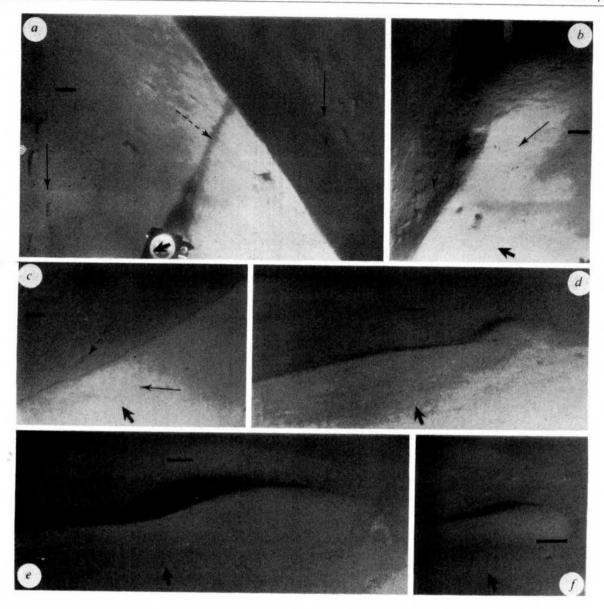


Fig. 2 Photographs of longitudinal triangular ripples. Bold arrow shows true north. a, LTR showing southwesterly flow within separation bubble on north flank (dashed arrow); main flow is to west (solid arrow). b, LTR formed behind biological mound; arrows as in a. c, LTR with cornice forming ripple crest. d, LTR developed behind biological mound. e, Mound at head of LTR, possibly of biological origin and current-modified; in d and e, changes in ripple-crest height give false appearance of sinuosity. f, Biological mound with well-developed tail, possibly precursor to LTR. a, b, and c are high-angle oblique photographs (courtesy C. D. Hollister); c, d, and e are low-angle oblique Troika photographs. Scale bars, 10 cm.

(Fig. 2d). In both cases fine current-produced lineations usually parallel the ripple crest. A third, common situation is a slightly asymmetrical crest (Fig. 2a, c). In such instances the fine-scale lineations define a short-term current trending at an angle to the crest (usually <45°), and a small 'slip-face' or cornice can be developed on the lee edge of the crest (Fig. 2c). On the lee flank of the ripple, the fine-scale lineations also trend upwards towards the ripple crest. The lee-side lineations indicate a zone of flow convergence; this convergence is thought to occur in a separation bubble because farther downstream from the ripple the lineations again parallel those on the upstream flank (Figs 2a, 3). Laboratory experiments by Allen (ref. 15, pages 210-216) show similar formation of a separation bubble behind skewed straight steps in a flume bed. When the incident current is at <40°-45° to the step (ripple) an open separation bubble is formed; it has helical internal flow (Fig. 3) that can extend beyond the tail of the ripple. A closed bubble, having internal circulation but lacking the extended helical flow, forms at greater angles of incidence (45°-135°).

Based on these observations, I propose that longitudinal triangular ripples originate as a consequence of four factors: (1) presence of a large (~10-20 cm high) seabed obstacle to flow; (2) bedload transport and/or deposition from suspended load; (3) presence of bottom currents with a significant mean flow; and (4) definite variation in direction of flow about the mean. In this model an obstacle to flow, such as a biologically produced mound, creates a leeward zone of flow convergence (wake); a tail oriented in the direction of mean flow is deposited in this wake. Common observation in beach and dune areas shows that beneath directionally uniform flows such a tail reaches only a finite size determined by the length of the wake behind the obstacle. However, where significant variations in flow direction occur, I suggest that a separation bubble is formed on one side or the other of the initial tail in response to the cross-bedform flow (Figs 2a, 3). The tail (ripple) thus is further constructed by bedload and/or suspended load transport towards the bedform crest on both sides of the ripple. This occurs in both closed- and open-bubble situations (any angle

of current incidence)15. However, at angles of current incidence <40°-45° an open separation bubble having helical flow should extend beyond the end of the bedform and facilitate elongation of the LTR through convergent bedload transport. As the LTRs propagate, the original flow obstacle may be modified and eventually be removed by erosion. As with the original moundand-tail, the LTRs parallel mean flow direction.

Of the four factors requisite to this model, the presence of a significant mean flow and superimposed variability are reasonably well documented from bottom photographs 16 and current measurements¹⁴. Current measurements at 5,022 m within the LTR area (Fig. 1) show a mean velocity of 28 cm s⁻¹ towards 253°, nearly parallel to the regional contours (~245°) over a 15-day period. Currents vary a total of about 110° in direction (190°-300°); for current speeds in excess of 50 cm s⁻¹, the total variation is about 60° (240°-300°). Longer-term records at 4,950 m (215 days) and 4,770 m (230 days) have considerably more variation in direction and lower maximum speeds (<40 cm s⁻¹), but they still show a significant mean velocity of 7-8 cm s⁻¹ directed towards about 250° (ref. 14).

The degree to which ripple construction results from bedload or suspended load transport is unclear. Bedload transport is suggested by better development of LTRs in the slightly coarser sediment on the south-west part of the Nova Scotian rise, but the texture and the LTR development could be independent responses to the particular current regime in this area. Asmechanism whereby suspended sediment is 'plastered' onto the bedform cannot be ruled out. Finally, the requirement for the presence of a flow obstacle to initiate LTR formation is supported by numerous photographs showing biological mounds at the head of LTRs as well as in varying states of elongation by currents (Fig. 2). I cannot absolutely define any of the time scales involved, but in light of the variability recorded in current measurements it is reasonable to suppose that the ripples take at least months to years to form beneath the mean flow and that the short-term variations (and small-scale lineations) occur over periods of days to weeks.

This model contrasts with one proposed by Flood⁷ for LTRs in the Blake-Bahama Basin. He suggested that LTRs may form by very rapid deposition beneath short-lived, high-velocity current events. This explanation is unlikely for the Nova Scotian continental rise because such current events commonly are not oriented parallel to the contours (deviations up to 55°, ref. 14) while the LTRs almost always are parallel. Furthermore, photographs from the Nova Scotian rise show that many LTRs consist of highly consolidated mud, often gouged at oblique angles by tool marks made by large bedload-transported particles. If the LTRs were evanescent bedforms, it is doubtful that they would achieve such strong consolidation. In the Blake-Bahama Basin, numerous biologically produced mounds of scale similar to the LTRs are observed in Flood's photographs, and the bedforms therefore may have an origin similar to those on the Nova Scotian rise.

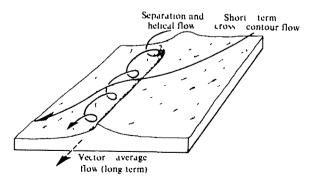


Fig. 3 Sketch showing propogation of LTR in direction of mean flow behind a biological mound. Instantaneous current oblique (<45°) to LTR and to mean flow stimulates helical circulation in separation bubble at left side of LTR. Alternations in current direction shift separation bubble between left and right sides of LTR, and they average to the long-term mean flow.

The qualitative model I have proposed does not deal with a host of important subtleties (such as the distribution of bed shear stress) in the nature of interaction between the boundarylayer flow and the bedforms. However, it presents a working hypothesis that agrees with available data and that can be tested by the future HEBBLE experiments and in laboratory conditions. The model predicts that longitudinal triangular ripples are characteristic of a specific set of flow conditions, namely significant short-term variability superimposed on a welldefined and geologically significant mean flow. Such conditions are most likely to be met on low regional slopes where deep boundary currents are established, especially along the western margins of ocean basins.

This work was supported by ONR contract N00014-79-C-0071 at Woods Hole Oceanographic Institution. I thank L. Sullivan and P. Bruchhausen for assistance with the photographic systems, which were supported by ONR Contract N00014-75-C-0210 at Lamont-Doherty Geological Observatory. I. N. McCave, D. G. Aubrey and C. D. Hollister made useful comments. Contribution no. 4984 of Woods Hole Oceanographic Institution.

Received 26 October 1981; accepted 17 February 1982

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Drifting buoy trajectories in the North Atlantic Current

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Despite intensive research, there are still large gaps in our knowledge of the North Atlantic Current which is considered to be the link between the Gulf Stream off the east coast of North America and the anomalously warm surface waters off northwestern Europe. In the classical scheme given in Fig. 1, which was constructed by Dietrich1 on the basis of data obtained during 1957-58, the current is seen to comprise several branches. Two important difficulties arise from this interpretation. First, the data for the area east of Newfoundland are not sufficient to define the causes of why the branching of the North Atlantic Current is stationary in its source area over the slope off Grand Banks2. Second, rapid surveys of the oceanic temperature structure by means of expendable sondes and, increasingly, of long-term moored instrumentation and satellitetracked drifting buoys3 have revealed that the oceanic current field is dominated by mesoscale eddies and meanders rather than by large-scale steady flows. We report here two experiments using satellite-tracked buoys which address these difficulties. Our results clarify the pathway of the North Atlantic Current and confirm that the current indeed consists of mesoscale eddies and meanders.

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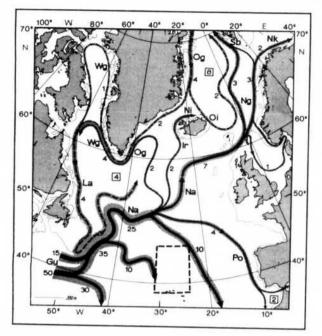


Fig. 1 The branching of the North Atlantic Current (Na) as inferred from the hydrographical data obtained during the International Geophysical Year 1957–58 (see refs 1, 2). The numbers indicate transports in the layer 0–1,000 m, given in $10^6 \, \mathrm{m}^3 \, \mathrm{s}^{-1}$. The dashed box depicts the area shown in Fig. 4.

In summer 1981, we carried out two experiments. The first was designed to verify the branching of the North Atlantic Current east of Newfoundland. Ten satellite-tracked buovs with drogues at 10 m depth were deployed by the Canadian RV Baffin between 5-8 and 14-16 May 1981 along two lines normal to the strong frontal zone south-east of Grand Banks. The lines were about 130 km apart. The buoys were launched at about 50 km intervals along these lines, starting in the zone of major horizontal gradients and running out into warm water. According to Fig. 1, this deployment would be predicted to release the buoys into the waters of the 'central' branch turning towards the Azores, into the 'northern' branch deviating into the southern Labrador Sea before crossing the Mid-Atlantic Ridge near 52° N and into the region of the anticyclonic gyre, located, according to Mann4 and Fig. 1, between the central branch and the Gulf Stream termination area.

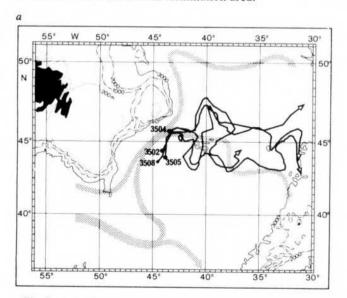


Figure 2a shows the trajectories of two groups of buoys. Drifters 3509 and 3507 were released at 12.2 and 10.0 °C sea-surface temperature in the most northwestern positions of the two launching lines. Both of them followed the northern branch of the North Atlantic Current; Buoy 3507 was trapped by the Flemish Cap for about 3 months before continuing northwards. The second group consisted of drifters 3500, 3501. 3503 and 3506, which were released at the most southeastern ends of the two launching lines at temperatures of 15.5, 16.5, 16.0 and 16.5 °C respectively. Buoy 3506 failed after a few weeks, while the other three drifters remained stagnant in the gyre area and could not escape until at least mid-September. The trajectories of a third group are shown in Fig. 2b. It includes buoys 3502, 3504, 3505 and 3508, which were deployed between the two groups shown in Fig. 2a at water temperatures of 15.3, 14.7, 16.5 and 16.8 °C respectively. All four buoys entered the central branch, and on nearing the Gauss Seamount group, two of them passed it to the north and two to the south. They continued to meander towards the Mid-Atlantic Ridge.

It is clear from Fig. 2a, b that the amplitudes of meandering and eddying are large enough for the trajectories of the different groups of drifters to overlap. For example, at around 2 June drifters 3504 and 3508 passed through the 'stagnation' area on their southern paths around the Gauss Seamounts; they had thus crossed the trajectory of drifter 3501 65 days earlier and would cross that of drifter 3500 8 days later. Drifter 3501 cut across the 1957-58 position of the current branch and the related front south-east of the Grand Banks without any significant change in the surface temperature readings. Sixty days later it also cut across the trajectories of the four drifters that finally followed the central branch. The existence of branches and a gyre (or stagnation area), as indicated in Fig. 2a, b, would suggest at least 200 km of spatial variability in the pathway of the current. A preliminary analysis suggests that wind did not significantly affect the trajectories during the observation period. First, the drogue-load sensors that were operational indicated no losses of drogues during the experiment. Second, winds were generally weak and variable during the first 2 months and even when they increased over the area between 30° and 50°W and 40° and 50°N from the south-west for two extended periods in late July and August, they did not cause a uniform displacement of the buoy field.

A few studies of the meandering and eddying of drifting buoys in the western³ and eastern⁵ Atlantic have provided evidence for a relationship between buoy trajectories and individual hydrographical features like Gulf Stream meanders or eddies and rings. We have now investigated this relationship

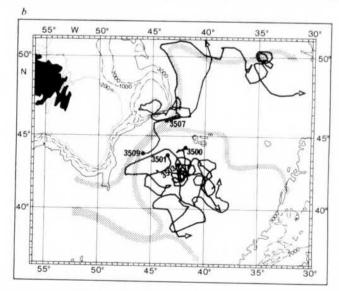
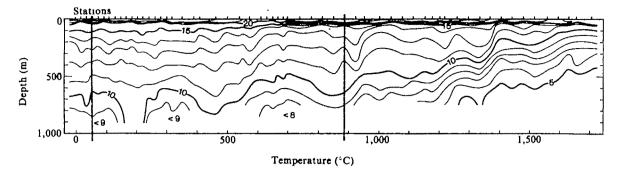


Fig. 2 a, b, Five-month trajectories of drifters drogued at 10 m depth and released mid-May 1981. The start of the trajectories is denoted by a dot and the number of the drifting buoy. The stippled areas correspond to the current branches shown in Fig. 1. Depths exceeding 3,000 m not contoured.



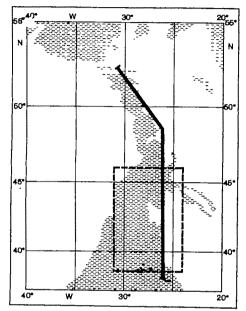


Fig. 3 Temperature section along the eastern flank of the Mid-Atlantic Ridge north of the Azores obtained on 12–16 August 1981. The portion of the section between the dashed lines corresponds to the meridional extent of Fig. 4. The small map shows locations in relation to the Mid-Atlantic Ridge (stippled area).

for a 700×400 km area over the Mid-Atlantic Ridge north of the Azores in August and September 1981. Figure 1 shows the location of this area between the northern and the central branch of the North Atlantic Current. A three-dimensional survey of the temperature and salinity fields was carried out at the same time as nine drifters drogued at 30 and 100 m depth were followed. The hydrographical setting in Fig. 3 shows the area to be dominated by mesoscale features. It also indicates that the drogues of the drifters were reaching down through the surface mixed layer. Figure 4 displays the depth of the 10 °C isotherm, which is representative of the depth of the main thermocline. It is based on 363 XBT and 49 CTD stations with an 'average' spacing of ~20 km. Four cold and three warm eddies have been found with an average diameter of 150 km. The trajectories of the buoys superimposed in Fig. 4 indicate a geostrophic flow around the eddies as a first approximation. The close correlation between the trajectories from the uppermost layers and the hydrographical structure at the depth of the main thermocline indicates that the eddy field dominates the flow in the warm water sphere. This result is confirmed by current meter records obtained by a moored vertical array. The current shear observed in the depth range from below the mixed layer to 662 m depth corresponds to that expected from the horizontal temperature gradients shown in Fig. 3.

It took 25 days to complete the hydrographical survey, including a repeat survey of the central and the northeastern region of the area shown in Fig. 4. As the trajectories presented in Fig. 4 are for a period 9 days longer, the eddy field must constitute a quasi-synoptic distribution. This is a basic requirement for most of the further analysis to be performed on this data set.

Another set of drifting buoys was released in August 1981 over the Mid-Atlantic Ridge between 46° N and Gibbs Fracture Zone at 52° N. It seems from the paths that an eddy field similar

to the one presented in Fig. 4 covers all this area. The buoys are still drifting across the North Atlantic and are expected to arrive off the the European coasts in spring 1982.

Drogued drifters provide a useful tool with which to investigate the kinematics of the current system, especially if simultaneous surveys of the hydrographical structures are possible. The extent to which satellite monitoring of sea-surface temperature pattern can help interpretation of buoy trajectories is not yet clear. IR satellite images of the region shown in Fig. 4 do not reveal any eddy field for the southern and the eastern

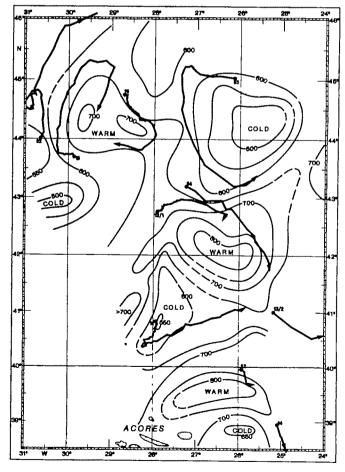


Fig. 4 The topography of the 10 °C isotherms and the trajectories of drifters drogued at 30 and 100 m depth. The start of the trajectories is denoted by a dot and the number of the drifter. The hydrographic survey lasted from 12 August to 6 September 1981. The trajectories are shown for the period 12 August to 15 September, with the exception of drifters 14 and 52, which started on 5 September. For general location of the area see Figs 1, 3.

part of the study area, although some features can be identified in the northwestern part.

We acknowledge the active participation of A. Clarke, E. Fahrbach, G. Hardtke, J. Stahlmann and A. Sy in this work.

Received 14 December 1981, accepted 1 March 1982.

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Cooperation and competition within coalitions of male lions: kin selection or game theory?

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Male lions form cooperative coalitions which compete against other coalitions for exclusive access to female groups^{1,2}. This cooperation and the apparently low level of intra-coalition competition over oestrous females, have been considered to be due to the close genetic relatedness of the males in the coalition¹⁻⁴. However, we now present evidence that breeding coalitions of male lions include non-relatives much more commonly than was generally supposed, that intra-coalition competition over females is widespread and that kinship is not the primary factor determining levels of competition.

Between July 1978 and May 1981 we studied the population of lions in the Serengeti, Tanzania, described by Schaller⁵, and all the lions resident on the floor of Ngorongoro Crater. Lion prides are stable social groups composed of 2-18 adult females, their dependent offspring and a coalition of 1-7 adult males (which can simultaneously control more than one group of females). Genealogical records have been maintained since 1966 for two prides in the Serengeti⁶ and since 1974 for another 13 prides in the Serengeti and Ngorongoro². We made daily censuses of as many prides as possible and recorded the incidence of fresh wounds, the consort partners and mating activity of each male, and the presence of non-consorting males ('rivals') within 200 m of each consort pair. We collected over 500 h of behavioural data on consort pairs during 2 h watches at dawn and dusk.

Theoretical analyses of male coalitions have assumed that they are always composed of relatives^{1,2}. However, there are examples of unrelated males becoming companions² data show that 42% of breeding coalitions of known origins

Table 1 The composition and origins of the 20 male coalitions having tenure in prides in the Serengeti and Ngorongoro during 1978-81

Coalition sizes	Origins
6, 5, 5, 4, 3, 2, 2 3 7, 4, 4, 4, 3 3, 2 3, 2, 2, 2	Unknown Pair of unknown origins with son Cohort from same natal pride Full siblings All unrelated Sibling pair with unrelated third

Males were considered relatives if they came from the same natal pride or joined their probable fathers, and non-relatives if they were known not to have come from the same natal pride or if they had associated with a variety of male partners for over a year before coming together (consistent with the behaviour of males known to be from different prides and completely unlike that of males of the same natal pride).

contained non-relatives (Table 1). This proportion is higher than the 10% reported by Bygott et al.2, because their data included cohorts that had not yet gained a pride (J. D. Bygott, personal communication). There is considerable mortality among subadult and nomadic males so that by the time they gain a pride their initial companions may have been lost and new ones found.

Competition for individual oestrous females between males from the same coalition consists primarily of competition for temporary 'ownership' of the female. When a female in a pride comes into oestrus, the first male that encounters her forms a consortship with her and this 'confers temporary dominance on the consorting male '5,6. The consorting male maintains proximity (usually <1 m) to the female, herds her and prevents other males of his coalition from moving too close to her. Behavioural oestrus (days with mating) lasts about 4 days. during which time copulation occurs once every 25 min; the inter-oestrus interval is about 16 days7. The male sometimes guards the female for 1 or 2 days before mating and for up to 6 days after mating has ceased. Males occasionally 'mistake' a potentially oestrous female, guarding her for a day or two and then leaving her after she fails to come into oestrus.

As both coalitions containing relatives and those containing non-relatives were common, it was possible to determine whether kinship affected intra-coalition aggression over oestrous females. Threats without contact by consorting males towards other males were common and occurred mainly when the other males were within 15 m of the consorting male's female (84%, n = 63 threats). Most of these threats (53%) were the direct result of the female attempting to move closer to rivals (or other consorting males). Kinship had no effect on the frequency of threats by consorting males to rivals (calculated per minute spent by a rival within 15 m of a consorted female and based on six consorting males whose unrelated companion(s) spent any time within 15 m of his consort partner, and on 12 males whose related companion(s) spent time within 15 m; U = 36, P > 0.50), or the time spent by rivals within 15 m of the consorted female (based on 10 males who had unrelated consorting companions, and 19 males who had related consorting companions; U = 76, P > 0.20). The fact that rivals spent any time as close as 15 m to the consort pair was due primarily to the behaviour of the female. Their presence at more moderate distances, however, depended on the behaviour of the rival, and rivals were more likely to be within 200 m of a consorted oestrous female as the availability of other possibly oestrous females declined (Fig. 1).

More serious intra-group fights over females included slapping and biting and frequently resulted in wounding of one or both males. Such fights were observed nine times and could be inferred another five times from fresh wounds on one or both males together with a change of consort partners. Serious fights were no more common between non-relatives than between relatives (P > 0.50); rather, they were context specific: of 13 fights where the context was known or could be inferred, eight occurred when 'ownership' of the female was undecided or unclear and four involved two consorting males. Ownership was 'undecided' when two males simultaneously came into the vicinity of an unconsorted, potentially oestrous female; and was 'unclear' when the consorting male moved further from the female than was the rival. There was usually a race between males to arrive first at a female. On arrival, the loser would defer to the winner though in subsequent consortships the previous consorting male might be a rival to the new consorting male (also see ref. 5). Lion males can consort simultaneously with two females and females being consorted by different males occasionally tried to come into close proximity to each other. In such cases one consorting male sometimes tried to take over the other's female.

Males also competed in a less direct manner to be the owner of a female when she came into oestrus. Males guarded nonoestrous females, probably in anticipation of the female's oestrus, and this was most common when the availability of oestrous females was lowest. When only one female in a pride was being consorted, the consorting male was more likely merely to be guarding a non-oestrous female than on days when several females were being consorted (T=40, n=19 males) observed consorting at least twice in each condition, P<0.05).

Males in Ngorongoro Crater guarded non-oestrous females more frequently than did males in the Serengeti. When only one female was consorted, the female was not in oestrus on 80% of occasions in the Crater (based on the behaviour of 11 consorting males) but on only 42% of occasions in the Serengeti (n = 24, U = 69, P < 0.025). Guarding of non-oestrous females may be more common in the Crater because it is less costly. Consort pairs rarely hunt and therefore feed infrequently⁵, but prey density in the Crater is so high and pride ranges so small⁸ that consort pairs can more easily join pride mates at kills.

Because fights over oestrous females are so rarely observed, males of a coalition have been said to 'share' females and to do so because they are relatives (see refs 1, 2). However, two factors should be taken into account when attempting to measure differential reproductive success among males of a coalition. First, females in a pride tend to come into oestrus at the same time^{5,6}. In our study, on the first day of 43% of oestrous periods other females in the pride were also in oestrus (n = 150). Second, females tend to move to additional mating partners after their first mating partner loses interest in them at the end of oestrus⁷. Subsequent partners show only a brief interest in the female and females seek additional partners most often when their fertility is lowest7. Thus calculating the relative mating activity of males without controlling for either the number of females in oestrus or the order in which males consorted with a particular female (as in refs 1, 2) biases against finding any differences in reproductive success.

The extent of differential male reproductive success was measured in 11 coalitions using data that eliminated these biases (Table 2). Coalitions consisting solely of relatives did not 'share' females more equally than did coalitions containing non-relatives. However, differences in competitive ability did result in differential male reproductive success. Disparities in consorting success were greater in coalitions in which the males were of markedly different ages or sizes than in those where all the members were evenly matched (Table 2). In all six of the former coalitions, the non-prime (either very young or old) and small individuals consorted less frequently with oestrous females than did their larger or more vigorous companions.

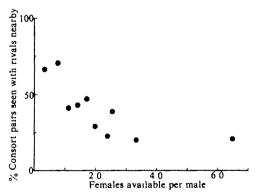


Fig. 1 The proportion of sightings (n=222) in which non-consorting males ('rivals') were seen within 200 m of consort pairs plotted as a function of the number of potentially oestrous females available to those males elsewhere (number of unconsorted females not pregnant or with cubs/number of non-consorting males). Only data taken from coalitions which had sole control of a pride are used and points were taken at least 4 days apart. Eight of nine coalitions showed trends similar to the overall result (P < 0.04) and there was no difference between the Serengeti and Ngorongoro, so all data were pooled and cells combined to give a $k \times r$ matrix with sufficient sample size to test for heterogeneity $(\chi^2 = 23.87, 9 \text{ d.f.}, P < 0.01)$. The trend for rivals to be present more often when fewer females are available elsewhere is significant $(r_s = 0.89, n = 10, P < 0.01)$.

Table 2 Extent of differential mating success in each coalition

Mating success of respective males	Includes non-relatives?	All same age and size?	I_{T}
3, 0, 0	Unknown	No	2.00
3, 0, 0	Unknown	No	2.00
5, 5, 0, 0, 0	Unknown	No	1.50
4,0	Yes	No	1.00
2, 0	Yes	No	1.00
3, 3, 0, 0	Unknown	No	1.00
7, 4, 3, 2, 2, 1, 1	No	Yes	0.47
7, 4, 2, 2	No	Yes	0.30
3, 2, 1	No	Yes	0.17
2, 1, 1	Yes	Yes.	0.13
2, 2	Yes	Yes	0

Each line gives data on a different male coalition. For each male in each coalition, the number of oestrous periods is given in which he consorted with a female who was the only oestrous female available to that coalition on the day when she was first observed mating. Only the first male to mate with the female in each of these periods is considered, unless a male was seen to win the female from another male in a fight-in these cases the victor was also included. Only coalitions in which the average number of such periods is one or more per male are included. For each coalition a measure of differential mating success, I_T (ref. 14), was calculated. Large values of I_T indicate unequal mating success; smaller values indicate evenly distributed mating success. Coalitions containing members of different size or age showed significantly more unequal mating success than coalitions with equally aged and sized members $(n_1 = 6, n_2 = 5, U = 0, P < 0.01)$, but there was no significant difference between coalitions consisting solely of relatives or including non-relatives $(n_1 = 3, n_2 = 4, U = 6, P > 0.50)$.

The high proportion of coalitions containing non-relatives is not surprising. Because singleton males almost never gain control of a pride and coalitions of three or more father more surviving offspring per male than do smaller coalitions², selection will favour singletons and pairs forming coalitions with additional males. As females are frequently in oestrus simultaneously, even subordinate males often have access to females.

Males would benefit even by cooperating with non-relatives but should prefer relatives as partners^{9,10}. We know of no case where a male with related companions left them for an unrelated companion, although there were cases where additional relatives were not accepted. A group of three relatives prevented three younger relatives from joining them, and incorporation of sons into the coalition was known or suspected only when the number of older males was one or two (n = 4 coalitions). In coalitions of three or more, incorporation of sons has never been seen, even though such large coalitions are more likely to maintain tenure long enough to father surviving sons² Because large coalitions can control prides without additional companions, they would be expected to exclude additional members when an added male would reduce each male's reproductive success (but not when the exclusion results in a loss in inclusive fitness). This is especially important if the new male would become disproportionately successful as in the case of ageing males being joined by males nearing their prime

Costs to males of direct competition for oestrous females can be high: one-to-one fights typical of such encounters often result in wounds to the face and eyes and sometimes in blinding⁵. Even in a gang attack on a single individual, the lone animal can wound several of its opponents. Furthermore, the loss of a companion through fighting may shorten tenure in the pride¹. Game theoretical analysis predicts that when costs of fighting are high, contests may be settled 'conventionally' through recognition of asymmetries such as 'owner versus rival' or 'large versus small', rather than through overt aggression 11-13. As differences in size or vigour exist in only about one-third of male coalitions (7 of 20), the 'respect' of ownership in lions is particularly important. Males were seen to compete for females indirectly by anticipating oestrus in a female in order to be the 'owner' when she eventually came into oestrus and they did

this more often when costs of guarding and availability of oestrous females were lowest. Fights were virtually restricted to occasions when ownership was unclear or when two consorting males were in close proximity.

In conclusion, the low levels of aggression observed within coalitions of male lions, which had been ascribed to kin selection, are not affected by the degree of genetic relatedness of the males and may be better understood in terms of game

We thank the H. F. Guggenheim Foundation, the Royal Society of Great Britain (to A.E.P.) and the National Geographic Society for support of fieldwork; the Government of Tanzania and the Serengeti Research Institute for permission and facilities; B. C. R. Bertram, J. D. Bygott and J. P. Hanby for data and for ensuring the continuity of the lion study; and J. Altmann, S. A. Altmann, S. J. Arnold, P. H. Harvey and J. Silk for comments. C.P. is supported by NIMH training grant MH15181.

Received 11 November 1981; accepted 12 February 1982.

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Properties of the Ca2+-activated K+ channel in one-step inside-out vesicles from human red cell membranes

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The presence of a calcium-activated potassium channel in some mammalian red cell membranes makes them useful experimental models in which to study the properties of similar mechanisms believed to be involved in the control of membrane potential and conductance, at rest and during activity, in many other cells. However, vesicles prepared from human red cell membranes by the method of Steck et al.1, whether inside-out (IOVs) or right-side out (ROVs), either failed to show any Ca2+-activated component of K+(Rb+) fluxes2 or showed only a very reduced calcium sensitivity and K⁺/Na⁺ selectivity³. This is surprising because there is good functional preservation of other transport mechanisms, such as the anion carrier⁴, Ca² pump⁵ and Na⁺ pump⁶. Our failure to confirm reports⁷ that the calcium response could be restored in 'silent' IOVs by addition of protein concentrates from red cell lysates prompted the search for and discovery of vesiculation procedures which produced ion-tight IOVs in a single step, with minimum loss of membrane components and transport properties⁸⁻¹¹. We report here (1) the conditions which favour preservation or loss of the Ca2+-activated component of the 86Rb efflux from one-step IOVs, (2) an approximate estimate of the number of Ca² activated K+ channels per red cell, and (3) that individual Ca²⁺-activated K⁺ channels respond in an all or nothing fashion to Ca2+ activation and differ in their threshold sensitivity to ionized calcium.

The IOVs used in these experiments were prepared by the one-step method and loaded with 86Rb+ as detailed in Fig. 1 legend. Preliminary experiments had indicated that when the one-step IOVs are resuspended in media which can sustain the activity of the Na⁺ pump (see Fig. 1 legend), the ⁸⁶Rb⁺ release caused by ATP addition exposes the total ⁸⁶Rb⁺ space sealed within IOVs that contain Na+ pumps. Figures 1 and 2 compare this Na⁺-pump IOV space with that accessible to Ca²⁺-activated channels, in conditions which optimally preserve (Fig. 1) or partially inhibit (Fig. 2) the Ca²⁺-dependent ⁸⁶Rb⁺ release.

In 11 experiments similar to that of Fig. 1, using red cells from three different donors, the ratio, r, of the 86Rb+ space accessible to Ca2+-activated channels to the 86Rb+ space accessible to Na+ pumps varied between 0.86 and 1.02. With an average of 500-1,500 vesicles of non-uniform size per cell¹¹ and 100-200 Na⁺ pumps per cell^{12,13}, the probability of a vesicle having more than one pump will be rather low. However, most of the ⁸⁶Rb⁺ released will be from the larger vesicles which contain a larger fraction of the ⁸⁶Rb⁺-sealed vesicular volume¹¹. These vesicles are more likely to have both pumps and channels, thus causing more overlap than expected had all the vesicles been of uniform size. If the surface density of pumps and channels is assumed to be uniform, the similarity of ⁸⁶Rb⁺ space accessible to Na+ pumps and K+ channels could reflect identity of the two mechanisms 14, structural linkage or simply numerical parity. The first two possibilities are unlikely because of the additional ⁸⁶Rb⁺-releasing effects of Ca²⁺ and ATP shown in Figs 1b and 3, which suggest that there are vesicles with either one or other of the two transporting systems. If the assumptions made here were correct, the number of Ca2+-sensitive K+ channels, like that of Na+ pumps 12,13, would be in the range of 100-200 per cell.

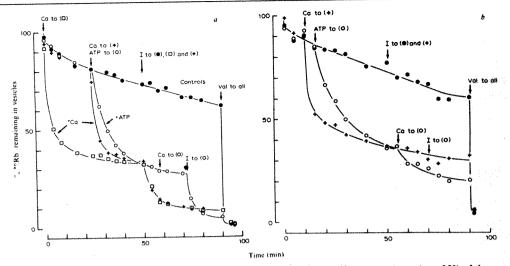
The experiment of Fig. 2 represents the conditions which cause relative channel inhibition. Lysis and vesiculation at high pH and in the presence of EDTA cause a variable reduction in r (0.06-0.65 range, in seven experiments similar to that of Fig. 2). As with the uninhibited channels (Fig. 1), the Ca2induced 86Rb+ release also saturated at about 50 µM Ca2+. Addition of concentrated protein extracts from red cell lysates or purified calmodulin15, as with Steck-type vesicles, failed to restore r and only had minute random effects. Partial inhibition, therefore, seems to represent irreversible channel inactivation. Low pH (7.5) per se had no effect on r. The critical condition for optimal channel inactivation seems to be enhanced divalent cation chelation by high pH (8.5) during vesiculation, as if the most vulnerable configuration were only transiently present at this stage. Higher EGTA or EDTA concentrations (0.5-3 mM) interfered with vesiculation itself and could not be tested. Used before or after, but not during, vesiculation, they caused only small and rather variable reductions in r even at pH 7.5, and, as before, only in the absence of added Ca²⁺ or Mg²⁺. These inhibitory effects of EGTA or EDTA at high pH could perhaps explain the failure to observe Ca2+-activated components of the K⁺(Rb⁺) fluxes in some of the Steck-type vesicle preparations2.

The protective effect of Ca²⁺ and Mg²⁺ suggests an interesting alternative interpretation of the Ca²⁺-sensitivity curves reported in resealed ghosts containing buffered calcium¹⁶⁻¹⁸. It may be that part of the reported Ca²⁺ affinity for activation of the channel represents Ca²⁺ affinity for protection against irreversible inhibition by EGTA. This interpretation is supported by preliminary experiments with resealed ghosts containing 1 mM EGTA and ⁸⁶Rb⁺. The Ca²⁺-dependent ⁸⁶Rb⁺ release was inhibited by up to 80% when Ca²⁺ was incorporated using the ionophore A23187 only during a final incubation relative to ghosts sealed from the beginning with similar buffered Ca2+ concentrations.

In 'one-step' IOVs, with partial or total preservation of functional Ca²⁺-sensitive K⁺ channels not able to be inhibited further, the true Ca2+ sensitivity of the K+ channels may be studied directly. As shown in Fig. 3, the striking finding was that with increasing Ca2+ concentrations, progressively more

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Fig. 1 Effects of ATP and Ca2+ on the Rb+ release from one-step human red cell IOVs. Red cells from freshly drawn blood were washed four times in about 8 vols of isotonic media containing NaCl+KCl, 150 mM, and Tris-Cl (pH 7.8 at room temperature), 10 mM, packed to about 80% haematocrit and lysed in 40 vols of an ice-cold medium containing Na-HEPES (pH 7.5), 2.5 mM, and Na-EGTA, 0.1 mM. After about 10 min, to allow for completion of lysis, 40-ml aliquots of this suspension were centrifuged for 10 min at 30,000g in the cold (4°C), the ghost pellets resuspended in 2-4 ml of the same lysing medium and incubated at 37 °C for 60 min. Spontaneous insideout vesiculation occurs during the first 15-20 min of this incubation, as described before9, but membrane elasticity increases with time11, allowing



subsequent tragmentation and rescaling of a fraction of the vesicles by vigorous passage through 27-gauge needles. Earlier measurements of $(Ca^{2+} + Mg^{2+})ATPase^{11}$ indicated that at least 25% of the original red cell membrane area surrounds IOV-sealed vesicular space. Traces of ${}^{86}Rb^+$ (10-50 μ Ci ml⁻¹) were added before (a) or after (b) vesiculation. Addition before vesiculation secures maximum ${}^{86}Rb^+$ trapping within the resealed vesicular space; after vesiculation, incorporation of ${}^{86}Rb^+$ was by needling. The 86Rb*-loaded vesicles (from 1 ml original 'packed' cells) were washed once (to remove extravesicular tracer), and resuspended in 8-12 ml of a medium containing (mM):NaCl, 50; KCl, 10; Na-HEPES (pH 7.5), 25; MgCl₂, 1.5; EGTA, 0.02 (medium 'A'). The 86Rb* remaining in the vesicles was measured, at the indicated times after the start of the final incubation (37 °C), in 0.1-ml aliquots of this suspension, run through Tris-neutralized Dowex 50-X-8 (200-mesh) columns contained within glass-wool-plugged Pasteur pipettes and eluted with 1.5 ml of ice-cold sucrose (200 mM) directly into counting vials. The results are expressed as per cent $^{86}\text{Rb}^+$ remaining in vesicles relative to the samples at t=0. The indicated additions were taken from concentrated stock solutions expressed as per cent — RD remaining in vesicles relative to the samples at t = 0. The indicated additions were taken from concentrated stock solutions (concentration in parentheses) to give the following final concentrations (mM): Na-ATP (100 mM), 1.5; A23187 (I) (2 mM in absolute ethanol) 0.01; CaCl₂ (30 mM), 0.07; valinomycin (Val) (1 mM in ethanol) 0.001. The final Ca²⁺ concentration of 0.05 mM used here elicited maximal ⁸⁶Rb⁺ release in all conditions (not shown). The average vesicle diameter was ~0.2 μ m. Addition of ionophore A23187 in the presence of Ca²⁺ revealed a substantial ⁸⁶Rb⁺ space contained within ROVs (a). This compartment did not incorporate much tracer by needling (b). Establishing the proportions of ROVs to IOVs by assaying acetylcholinesterase activity as in multistep vesicles, does not give reliable values in one-step IOVs because vesiculation is performed without washing and the enzyme may easily reassociate with both sides of the membrane. Ca²⁺ addition after ATP-induced ⁸⁶Rb⁺ depletion had no measurable effect in a but caused further ⁸⁶Rb⁺ loss in b. In a, where all the resealed vesicular space was labelled, a disproportionate fraction of this space may be contained within the larger vesicles. Their chance of having more than one pump or channel may be higher than in the smaller labelled vesicles of b and this may explain the larger overlap of *6Rb* space

available to both mechanisms without necessarily postulating identity or link (see text).

vesicles released their 86Rb+ at the same maximum relative rate, instead of, as expected, all the channel-containing vesicles releasing their 86Rb+ at increasingly higher rates. The ratio between the rate measured over the first 5 min and the steadystate difference remained virtually constant at all Ca2+ concentrations (see Fig. 3 inset).

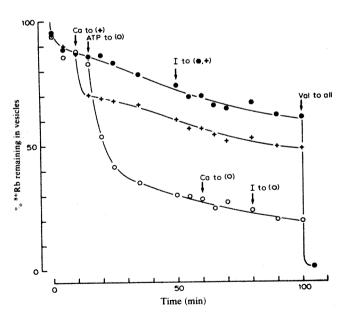


Fig. 2 Inhibition of Ca2+-induced relative to ATP-induced 86Rb+ release in one-step human red cell IOVs. This experiment was identical to that in Fig. 1a except that lysis and vesiculation were performed in a medium containing (mM): Na-HEPES (pH 8.5), 2.5, and Na-EDTA, 0.1. Possible inhibition of the Ca-sensitive channels in the ROVs could explain the failure of A23187 to activate further 86Rb+release as in Fig. 1a. In any case, the contaminant ROV space in this experiment cannot exceed 22% of the total **Rb*-resealed vesicular space because at least 78% of it must be contained within IOVs with Na+ pumps.

This means that Ca2+ activation is all or nothing for each channel and that the channels differ in their threshold Ca2+ sensitivity. The insert in Fig. 3, therefore, does not report any true or apparent Ca2+ affinity, but the distribution of Ca2+ thresholds in the channel population of this vesicle preparation. In three other similar experiments, there was some variation in the threshold distribution curve, with channels still being activated in the 2-10 µM Ca2+ range, but always in an all or

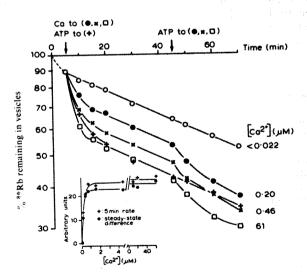


Fig. 3 Ca²⁺-sensitive ⁸⁶Rb⁺ loss from one-step IOVs as a function of the concentration. IOVs were prepared as in the experiment of Fig. 1a. The final suspending medium ('A') had a contaminant Ca²⁺ concentration (as determined with Ca²⁺ electrodes) of 7 and 100 µM of added EGTA. CaCl₂ was added to give the calculated concentrations of Ca²⁺ shown. ATP addition as in the experiment of Fig. 1. In three other experiments using medium A and 1 mM EGTA, the threshold distribution curve (see inset) was shifted slightly to the right (app $K_1 = 2 \mu M$). Ca²⁺ uptake by the IOVs before ATP addition was less than 2% of the total Ca²⁺ even at the lowest Ca2+ concentration (not shown). This rules out Ca2+ depletion through vesicle binding as the possible cause of the all or nothing response.

nothing fashion. Apparent changes in Ca2+ sensitivity therefore result from shifts in the threshold distribution curve.

If the all or nothing response of each channel reflects the behaviour of the channels in the original cells, a graded increase in K⁺(Rb⁺) flux in intact cells or resealed ghosts, in conditions where the Ca²⁺ concentration is uniformly raised in all the cells, must reflect either activation of only a fraction of the channels in each cell or, if all the channels belonging to the same cell had similar Ca²⁺ thresholds, activation of all the channels in only a fraction of the cells. It is unlikely that cells with few or no channels contribute to the graded response because, when Ca²⁺ is incorporated at high ionophore (A23187) concentrations in intact cells, the entire cell K⁺ pool participates in the ⁴²K⁺-tracer equilibration process¹⁹. Tracer K⁺(Rb⁺) pools retained within cells with inactive channels have been observed

Received 24 September 1981, accepted 25 February 1982

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in intact cells and resealed ghosts²⁰⁻²⁴, although seldom in conditions of known uniformity of Ca²⁺ or Pb²⁺ (ref. 20) content. Such all or none cell responses^{20,24}, however, do not imply all or nothing single channel behaviour as found here, because they can also result from variations in the Ca2+ sensitivity of channels belonging to different cells, each channel responding to Ca²⁺ in a graded manner.

Too little precise information exists at present to define the range of possible kinetic models which could explain the present observations. Sharp all or nothing responses at a molecular level may require interacting configurational changes, occluded states or dCa2+/dt-sensitive gating mechanisms.

We thank J.D. Cavieres, R. W. Tsien, R. M. Bookchin and I.M. Glynn for helpful discussions, Mrs J. Gray for technical assistance and the MRC and EMBO for funds.

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All-or-none response of the Ca²⁺-dependent K⁺ channel in inside-out vesicles

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In several cell lines, an increase in the intracellular Ca2+ concentration elicits an increase in the membrane permeability to K⁺(Rb⁺) by activation of the so-called Ca²⁺-dependent K⁻ channel. The human red cell has been widely used to study this transport system². As Ca²⁺ (refs 3, 4) and other possible modulators^{5,6} act on the intracellular side of the membrane, a preparation of everted vesicles would be most useful for investigating such actions. Although several previous studies in inside-out vesicles have been unsatisfactory 8-10, a new vesiculation procedure that preserves Ca^{2+} -dependent K^+ transport has recently been reported 11,12. Using this preparation we now find that each vesicle responds to Ca2+ in an all-or-none fashion, the number of activated (permeable to ⁸⁶Rb) vesicles increasing with Ca2+ concentration between 10-8 and 10-6 M. This behaviour probably reflects the all-or-none response of individual K^+ channel units. The sensitivity to Ca^{2+} is decreased by Mg2+

⁸⁶Rb fluxes were measured at room temperature and in equilibrium exchange conditions. In vesicles loaded with ⁸⁶Rb during the vesiculation step, the addition of Ca²⁺ to the medium elicited a rapid release of ⁸⁶Rb, the efflux rate constant returning close to control values (without Ca²⁺) after 10-20 min. The fraction of vesicular space emptied of ⁸⁶Rb during this rapid phase increased in a graded stepwise fashion with increasing Ca²⁺ concentrations, being maximal at 10⁻⁴ M (Fig. 1a). A similar behaviour was observed for the uptake of ⁸⁶Rb (Fig. 1b). Both the transient nature of these rapid 86Rb fluxes and the graded response suggest that increasing Ca2+ concentrations activate an increasing number of vesicles, each vesicle responding in an all or nothing fashion with a fast and full equilibration of ⁸⁶Rb. Time-dependent inactivation of the K⁺ channel was excluded because vesicles loaded with ⁸⁶Rb by a 15-min incubation in the presence of submaximal concentrations of Ca2+ released most of their radioactivity when transferred to tracerfree medium containing the same Ca2+ concentration. On the other hand, if the vesicles were loaded at a Ca2+ concentration giving maximal response (10⁻⁴ M) and then transferred to medium containing different submaximal Ca2+ concentrations, there was a graded stepwise release similar to that shown in Fig. 1.

The simplest explanation for the graded vesicular response is that the different vesicles have different threshold sensitivities to Ca²⁺. However, the same response would be expected for a vesicle population of uniform sensitivity responding at random if the state of each unit (active or inactive) cannot change as long as the Ca2+ concentration remains unmodified. A kinetic model that could explain the all or nothing response of the K+ channel has been proposed by Lew, Muallem and Seymour (see accompanying letter¹³).

Our measurements of the space resealed to 86Rb after vesiculation show that each red cell should provide 200-300 vesicles. Assuming that the number of K⁺ channels per cell is less than 200 (ref. 12), the mean frequency of channels per vesicle should be below 1. In agreement with this prediction, we find that a substantial fraction (15-40%) of ⁸⁶Rb loaded during the vesiculation step is not released by Ca²⁺, even in the presence of the ionophore A23187, which renders right-side out vesicles sensitive to Ca2+. It thus seems reasonable to assume that most of the Ca2+-sensitive vesicles should have only one or a few channel units. On these bases, the all-or-none nature of the vesicular response does probably reflect the behaviour of individual channel units.

The Ca²⁺-induced fluxes were specific for Rb (²²Na fluxes remained unchanged) and sensitive to quinine (92-97% inhibition at 1 mM) at Ca²⁺ concentrations ≤ 10⁻⁶ M, properties similar to those described in intact cells or resealed ghosts3,14-18. However, at 10^{-4} M Ca²⁺, we found a quinine-insensitive increase of the fluxes of ²²Na (to twice the basal value) and ⁸⁶Rb (see the difference between the values at pCa 4 and pCa 6 in Fig. 2). This observation suggests a nonspecific effect of Ca2+ at such concentrations and is reminiscent of the simultaneous increases of Na+ and K+ permeabilities reported previously in red cells after treatments that presumably increase their intracellular Ca2+ content19

The study of the kinetics of the Ca²⁺-induced ⁸⁶Rb fluxes was complicated by their rapidity $(t_{1/2})$ of ~ 0.5 min) and also by the fact that they could not be described by single exponentials. However, the fraction of the vesicular space equilibrated with the incubation medium at the end of the rapid phase for a given Ca²⁺ concentration was highly reproducible between experiments if expressed as the per cent of the maximal effect. In addition, these values were similar for the uptake and release experiments. The activation of ⁸⁶Rb fluxes began at 10⁻⁸ M Ca²⁻¹ and was almost complete at 10⁻⁶ M Ca²⁺, half-maximal activation occurring at a pCa of ~ 7.55 (Fig. 2).

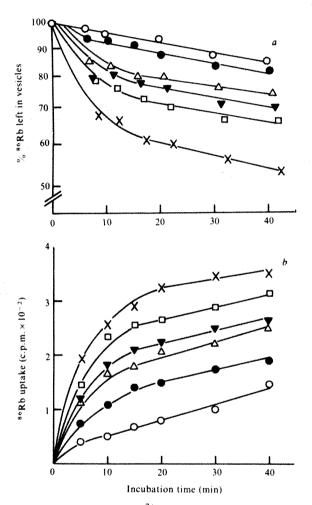


Fig. 1 Effects of external Ca^{2+} on the release (a) and uptake (b) of ⁸⁶Rb by inside-out vesicles. Vesicles prepared as described by Lew and Seymour ¹² were needled, washed once and resuspended in medium of the following composition (mM): KCl, 18; EGTA, 0.1; K-HEPES, 16.5, pH 7.5. For the transport experiments, 0.2-ml aliquots of vesicle suspension (corresponding to ~ 0.2 g of initial cells) were mixed with 0.6-1 ml of medium. 86Rb was added either to the vesiculation medium (release experiments) or to the final incubation medium (uptake experiments). Fixed Ca² centrations were obtained using mixtures of EGTA or Nhydroxyethylethylenediamine triacetic acid (HEDTA) (final concentration 0.4 mM) and calcium. Dissociation constants, measured in the incubation medium²⁰, were: EGTA, 4×10^{-8} ; HEDTA, 4×10^{-7} . The incubation period was terminated by running 0.1-ml aliquots of the vesicle suspension through 1-ml columns containing Dowex 50-X8-100 resin 12.21. Radioactivity was measured by Cerenkov counting. Symbols represent the following pCa values: ≤9 (○), 7.85 (●), 7.55 (△), 7.37 (▼), 6.88 (□), 4.00 (×). Vesicular space resealed to ⁸⁶Rb (ref. 12) ranged over 76–130 μ l per g of cells in different experiments. 40-70% of that space was sensitive and this value increased to 60-85% in the presence of 2 μM A23187. Everted vesicular surface was 25-35% of the total.

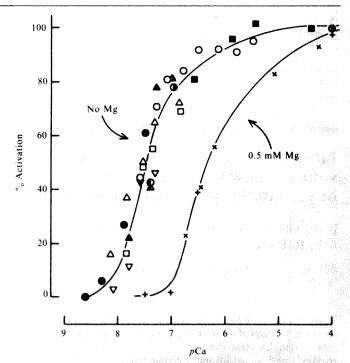


Fig. 2 Activation of ⁸⁶Rb transport by Ca²⁺. The vesicular space sensitive to Ca²⁺ (that which equilibrates with the medium during the rapid phase) was calculated from experiments similar to those shown in Fig. 1 by extrapolation to zero-time. Per cent activation was then calculated taking the value obtained without Ca2+ as 0% and that obtained at pCa 4 as 100%. The results obtained in 14 experiments with vesicles prepared using different blood samples are shown with different symbols. Squares represent data obtained in release experiments while all other symbols correspond to uptake experiments. The curve on the left was obtained in the absence of Mg^{2+} and the one on the right with 0.5 mM Mg^{2+} .

The procedure described here offers an easy way of investigating modifications of the sensitivity to Ca2+ of 86Rb transport. The addition of 5×10⁻⁴ M MgCl₂ to the medium reduced the apparent pCa value at which half-maximal activation of ⁸⁶Rb transport took place (pCa_{1/2}) from 7.55 to 6.35 (Fig. 2). Smaller Mg²⁺ concentrations $(2 \times 10^{-5}, 5 \times 10^{-5}, 10^{-4}, 2 \times 10^{-4} \text{ M})$ produced smaller variations of pCa_{1/2} (7.35, 7.15, 6.95, 6.82), a 10-fold increase of Mg²⁺ concentration decreasing pCa_{1/2} by 0.58 units. The inhibition of Ca²⁺-dependent K transport by Mg²⁺ reported here is consistent with previous observations in resealed ghosts¹⁵. The actions of other intracellular effectors on the sensitivity to Ca2+ of K+(Rb+) transport could be investigated similarly. Experiments performed in our laboratory have shown, for example, that neither calmodulin nor NADH modifies the kinetics of activation by Ca2+ nor the inhibition by Mg²⁺ or quinine. Work is now in progress to investigate the effects of other intracellular proteins and reducing agents.

We thank Dr V. L. Lew for valuable discussions and J. Alvarez for technical assistance. This study was supported in part by a grant from the Fondo Nacional para el Desarrollo de la Investigación Científica.

Received 3 December 1981; accepted 1 March 1982.

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Intracellular Ca2+activates a fast voltage-sensitive K+ current in vertebrate sympathetic neurones

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Many neurones, when depolarized, exhibit two components of outward K+ current—the voltage-sensitive delayed rectifier current originally described in squid axons by Hodgkin and Huxley¹, and an additional current triggered by the entry of Ca^{2+} ions². These two currents have been termed I_K and I_C , respectively³. Previous experiments have indicated that both forms of K^+ current are also present in vertebrate sympathetic neurones⁴⁻⁶. We have now studied the properties of $I_{\rm C}$ in bullfrog sympathetic neurones, uncontaminated with I_{K} , by injecting Ca2+ ions into the cells and measuring the resultant outward currents under voltage-clamp, in the manner previously used for large molluscan neurones^{7,8}. We find three interesting properties of $I_{\rm C}$ in these vertebrate neurones. First, it shows strong voltage sensitivity independent of the voltage sensitivity of the Ca2+ channels (which have been bypassed by the injection technique). Second, I_C activates and deactivates very rapidly ($\tau_c \le 20 \text{ ms}$ at 0 mV), with stepped changes in membrane potential. Current fluctuation analysis and patchclamp records of single-channel currents yielded evidence for appropriate short-lifetime ionic channels with a maximum conductance of $\sim 100 \text{ pS}$. Finally I_{C} in ganglion cells is highly sensitive to external tetraethylammonium. We deduce that in these neurones I_C is a fast current which can contribute a substantial fraction to the repolarizing current during an action

Figure 1a illustrates outward currents evoked in a voltageclamped bullfrog sympathetic neurone by brief iontophoretic intracellular injections of Ca2+ ions at different holding potentials. The first two panels show currents produced by equal negative and positive iontophoretic pulses at the initial holding potential of -30 mV. There is no change in current-to-ground (upper trace) during the negative iontophoretic pulse because the iontophoretic current was balanced by positive clamp current and no additional active membrane current was induced. However, during positive iontophoresis an unbalanced outward membrane current appears, outlasting the iontophoretic injection. With such brief (<1 s) iontophoretic applications, repeatable currents could be recorded at 5-10-s intervals without decrement. At any given holding potential the peak outward current increased linearly with increasing iontophoretic currents up to 10 nC. However, the sensitivity varied greatly from cell to cell: it was usually within the range 1-3 nA outward current per nC injected current at -30 mV, but some cells showed no response to 10 nC or more. Assuming a transport number of 0.3 for Ca^{2+} in 0.2 M $CaCl_2$ solution, 10 nC current should expel ~1.5×10⁻¹⁴ mol of Ca^{2+} ion; in a spherical cell of

 \sim 40 µm diameter this is equivalent to an increment of \sim 0.5 mM internal [Ca²⁺], although the increase in free ionized [Ca²⁺] is presumably much less because of buffering and redistribution. Unlike the situation in large molluscan neurones⁷, vertical movement of the iontophoretic pipette did not greatly alter the magnitude or time course of the current response, suggesting that in the smaller ganglion cells the intracellular Ca2+ concentration very rapidly attained homogeneity. Control injections to ± 10 nC from electrodes filled with 0.3 M KCl at pH 5.8 did not produce outward membrane currents.

The outward current generated by constant iontophoretic Ca²⁺ injections increased steeply as the membrane was depolarized between -50 and -20 mV, as shown on the right of Fig. 1a. In normal Ringer solution the current fell to zero around -50 mV and did not reverse with further hyperpolarization. Clear reversal to an inward current (at -35 ± 2 mV (\pm s.e.m.); n = 8) was obtained on raising [K⁺]_{out} to 25 mM. Assuming the underlying conductance change G_c at any membrane potential to be unaffected by raising [K⁺]_{out}, the hypothetical reversal potential in normal Ringer solution (containing 2.5 mM K⁺) could be calculated from the ratio of the currents evoked at a fixed holding potential in normal and high [K+] solutions and from the reversal potential in high [K⁺]. The mean extrapolated reversal potential in normal Ringer was estimated at -78± 7 mV (n = 5), and the positive shift on raising [K⁺] 10-fold was $+41 \pm 7 \, \text{mV}$.

At a reversal potential of -78 mV, the voltage dependence of $I_{\rm C}$ shown in Fig. 1a accorded with an exponential increase in the underlying conductance ($\Delta G_{\rm C}$) for each 7.5 mV depolarization above -50 mV. In seven experiments of this type, the average slope was 11 mV per e-fold $\Delta G_{\rm C}$ up to -10 mV. This is appreciably steeper than that previously reported (25 mV (ref. 8) and 28 mV (ref. 10)) for the voltage-sensitivity of $I_{\rm C}$ activated by intracellular Ca²⁺ injections in molluscan neurones. Removal of external Ca²⁺ or addition of the Ca²⁺-channel blocker Cd²⁺ in concentrations (0.2-0.5 mM) sufficient to block the inward Ca2+ current in bullfrog sympathetic neurones11 did not materially change this voltage sensitivity, showing that it was related solely to the action of internal Ca²⁺ and not to any inward Ca²⁺ current. Steady outward currents at depolarized potentials were reduced in Cd2+ solution, which allowed the membrane potential to be held at more positive potentials than in normal Ringer solution. This revealed that, at potentials positive to $-10 \,\mathrm{mV}$, I_{C} tended to saturate and, in some cells, actually diminished with increasing depolarization. This could not be alleviated by reducing the iontophoretic Ca2+ current.

When a voltage step was applied during a Ca²⁺ injection, the outward current showed an exponential growth or decline with time, and a time constant of ~15 ms at -10 mV command potential (Fig. 2a). To study these relaxations in more detail, the single microelectrode clamp method (which had limited current-passing capacity) was replaced with a conventional twoelectrode clamp⁹ and I_C was generated either by Ca^{2+} injection or by activating a sustained inward Ca²⁺ current with a long depolarizing command. Figure 3 shows examples of such depolarizing commands, in another context. During sustained depolarization the delayed rectifier current becomes totally inactivated so that, if no Ca²⁺ is present in the external solution, only a leak current is recorded and a superimposed voltage step produces a square 'ohmic' current response (Fig. 2b, lefthand record). When Ca2+ is added to the external solution the inward Ca^{2+} current generates an additional outward I_C (which is blocked by Cd2+) and a voltage step now produces a timedependent relaxation comparable with that seen during a Ca2+ injection (Fig. 2b, right panel). The average time constant for this relaxation (τ_c) was 16.0 ± 0.9 ms (n = 10) at -10 mV. Over the range -80 to +30 mV, τ_o showed an exponential lengthening for each 27 mV depolarization. An equivalent voltage sensitivity of these relaxations was confirmed when Ca²⁺ was injected through a third intracellular microelectrode.

The current record during long depolarizations in Ca²⁺ solution was distinctly 'noisy'. This noise disappeared on removing

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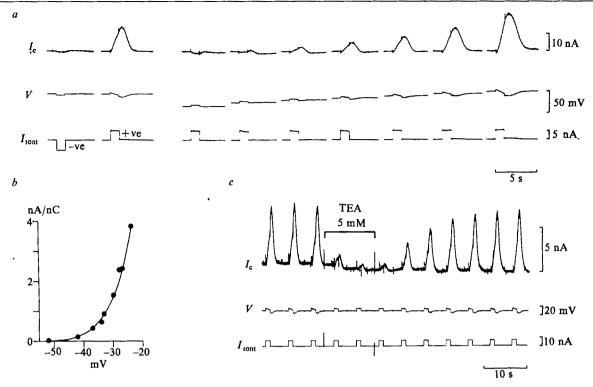


Fig. 1 Outward currents generated by intracellular Ca²⁺ injections in voltage-clamped bullfrog sympathetic neurones. Lumbar sympathetic neurones were maintained at 22 °C in normal Ringer solution containing 2.5 mM KCl, 2 mM CaCl₂, 10 mM MgCl₂ and 2 mM Tris buffered to pH 7.2 with HCl. Neurones were impaled with two electrodes drawn from thin-walled glass tubing (tip resistances 8-20 MΩ). One electrode, filled with 0.2 or 0.4 M CaCl₂ solution in distilled water (pH 5.8), was used to record membrane potential and to inject clamp current via a sample-and-hold voltage-clamp preamplifier (Dagan 8100, switching between voltage sampling and current injection at 3 kHz). Clamp current (upper trace m a and c, I_C) was recorded as true current-to-ground, filtered at 300 Hz; iontophoretic current (lower trace in a and c, I_{Cm}) was recorded by subtracting Dagan current (continuous output) from current-to-ground. The records in a show responses to 1 s, 6 nA iontophoretic injections of Ca²⁺ at different membrane holding potentials between -55 and -20 mV, preceded by test responses to negative and positive iontophoretic currents at -30 mV holding potential b, Shows the change in peak outward current with holding potential. The potential on the abscissa is the recorded membrane potential at the height of the current response. The smooth curve shows how the current would change if the underlying conductance increased e-fold for 7.5 mV depolarization and holding potential of -30 mV.

external Ca2+ or adding Cd2+. Spectral analysis of the Ca2+dependent noise during the latter stages of the depolarization (when the a.c. current record was flat) yielded Lorentzian power spectra (Fig. 3b); spectra obtained at rest, during hyperpolarizing commands or during depolarizations in the absence of Ca2+ or presence of Cd²⁺, always resembled the background spectrum shown in Fig. 3b. The average corner frequency (f_c) of the Lorentzian spectra at a command potential of -10 mV was 7.9 ± 1.5 Hz (mean \pm s.e.m.; n = 6). This corresponds to a time constant ($\tau = 1/2\pi f_c$) of 20 ms (16.9–24.9 ms), which is similar to that for the current relaxation following a voltage jump to -10 mV. Assuming the fluctuations to arise from transient openings and closings of many individual ionic channels, each with a fixed conductance (see below), the average single-channel current can be estimated from the ratio of the total variance to the mean current¹². Total variance was calculated from the area under the test spectrum between 0.1 and 200 Hz, after subtraction of the background spectrum. The average value of the macroscopic current was taken as the median value during the spectral computation—a reasonable approximation for long depolarizations and near-linearly decaying currents. Mean Ic was determined by subtracting the current during equal but opposite hyperpolarizations, or during depolarization in the presence of Cd²⁺ or absence of Ca²⁺. (Residual currents were rather small, and imprecision in the measurement of mean $I_{\rm C}$ contributed greatly to the scatter on the resultant ratios.) In one cell clamped to 0, +10 and +20 mV, the variance increased linearly with the mean current. This suggests that the probability of channel opening was relatively low, so that the non-stationary currents would not introduce significant distortion into the spectra if analysis is restricted to the period when the a.c. current record is flat¹³, as in our experiments. The mean ratio of variance to mean I_C was 8.1 ± 1.7 pA (mean \pm s.e.m.; n = 17 cells), giving

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an average single-channel conductance of $\sim 100 \pm 20$ pS at a reversal potential of -78 mV.

In ganglion cells cultured for 1-2 months in vitro 14 (which conveniently had naked cell membrane), appropriately large single channels could be detected using the gigohm-seal patchclamp technique^{15,16}. An example is shown in Fig. 4. At rest potential (around -60 mV in the unimpaled cells, judging from the better values obtained on impalement) the current record was quiescent. When the electrode was polarized, thus depolarizing the enclosed membrane patch, one or more discrete current channels appeared whose opening frequency and duration clearly increased with increasing depolarization. During weak depolarization the channels showed simple openclosed behaviour, but at depolarization ≥80 mV the open state was interrupted by brief closures. These became more frequent with increasing positivity, giving a very noisy open-state current level. Up to 100 mV depolarization the single-channel current increased linearly with applied potential, with a slope corresponding to ~100 pS open-channel conductance. Similar values were obtained in two other patches. However, at more positive potentials this linearity was lost and at ≥175 mV the peak single-channel current actually declined: such behaviour was seen in one other patch which could be polarized sufficiently. At 60 mV patch potential (~0 mV membrane potential) the mean channel open time was ~20 ms. The apparent open time increased exponentially per 22 mV depolarization up to +150 mV patch potential.

These single-channel events are clearly similar to those recently described in isolated mammalian chromaffin cells¹⁷ and cultured embryonic muscle cells¹⁸, in terms of both the large single-channel conductances and the open state flutterings. The latter may result from superimposed channel blocking¹⁶ or might relate to the vibrations between open and closed states

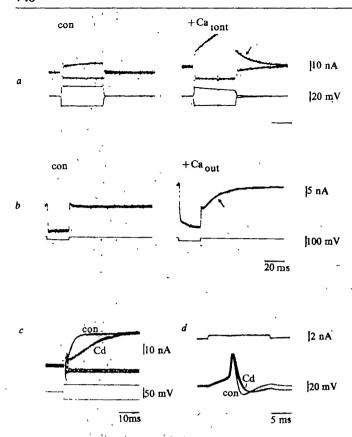


Fig. 2 Kinetic tests on $I_{\rm C}$. a, Voltage jumps of +10 mV and -10 mV applied from a holding potential of -10 mV before (con) and during (+Ca_{lout}) intracellular iontophoresis of 1 nA Ca. The single microelectrode voltage clamp technique referred to in Fig. 1 was used to record voltage and pass current: the limited current-passing capacity leads to some clamp failure during the large outward current but not during the repolarizing tail current (arrowed). b, 30 mV hyperpolarizing voltage jumps applied from a steady prepotential of -10 mV in Ca²⁺-free solution (con) and in 2 mM Ca²⁺ solution (+Ca_{out}). Two-electrode voltage clamp, no Ca²⁺ mjection. Note the relaxation (arrowed) in the presence of Ca²⁺. c, Voltage commands of ±40 mV applied from a holding potential of -50 mV in normal Ringer solution (con) and then in Ca²⁺-free solution containing 0.5 mM Cd²⁺ (Cd). Two-electrode clamp. d, Action potentials generated by depolarizing current injections in normal Ringer solution (con) and in Ringer containing 0.5 mM Cd²⁺ (Cd), recorded using independent voltage-recording and current-passing electrodes.

seen in n-acetylgramicidin channels¹⁹. As in the chromaffin cell experiments¹⁵, it was not necessary to have Ca^{2+} in the pipette to observe channels, so presumably there was sufficient free Ca^{2+} inside the cell to trigger them. Single I_C channels have also been detected in molluscan neurones²⁰, of conductance (19 pS) rather lower than but mean open times comparable with those in the ganglion cells, and in neuroblastoma cells (C. F. Stevens, personal communication).

As the cultured cells showed macroscopic C-currents of similar amplitude, time constant and voltage sensitivity to those in freshly dissected ganglia, we suppose that the single channels represent the subunits of these macroscopic currents. If this is so, the comparable duration and voltage sensitivities of the channel open times and relaxation times suggest that the latter are determined primarily by the channel closing rate. This implies that a relatively small proportion of the available channels was activated by the intracellular Ca2+ injections or by the imposed depolarizations. The fall in single-channel conductance at very positive potentials might explain the saturation of the macroscopic current with increasing depolarization. However, we also detected signs of a loss of voltage sensitivity at high internal Ca2+ concentrations. This was predicted from the observations on chromaffin cell channels¹⁷, where large or repeated Ca²⁺ injections abolished the relaxations previously generated by voltage jumps. The origin of the voltage sensitivity is not clear: voltage dependence seems too steep to be accounted for entirely by voltage-sensitive binding of Ca2+ to a site within the membrane potential field⁸, unless the binding of more than one Ca²⁺ ion per channel was necessary²¹ or an additional energy barrier were imposed²²; alternatively, voltage sensitivity might be due to the gating of the K+ channels themselves.

Two further points of interest concerning ganglionic C-channels relate to their pharmacology and physiological function. First, like some 23,24 but not all 3,25 molluscan neurones, $I_{\rm C}$ in these ganglion cells was at least as sensitive as the delayed rectifier current to external tetraethylammonium (TEA). Thus, the currents generated by ${\rm Ca}^{2^+}$ injections (Fig. 1c) or by membrane depolarization were virtually abolished by 5 mM TEA, and were half-reduced at 1 mM. In agreement with this, TEA suppressed both voltage-jump current relaxations and current noise. Thus, the effect of TEA on these neurones cannot be attributed unequivocally to inhibition of delayed rectifier currents. In contrast, $I_{\rm C}$ was insensitive to 1 mM external 4-aminopyridine.

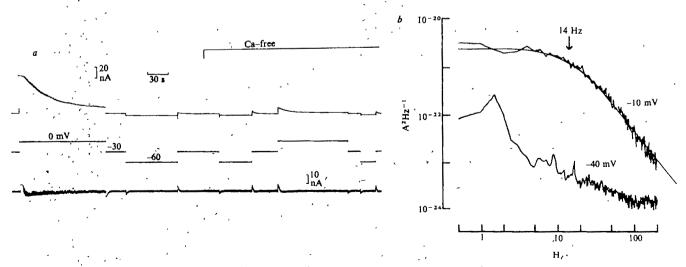


Fig. 3 Current noise associated with I_C recorded from freshly dissected ganglion cells with a two-electrode voltage clamp. The records in a show currents evoked by long ± 30 -mV commands from a holding potential of -30 mV in normal Ringer solution and after replacement with Ca^{2+} -free Ringer. Upper trace, d.c. current; middle trace, voltage; lower trace, a.c. current (0.1 Hz high-pass filter). Note that, in the absence of Ca^{2+} , the delayed rectifier current evoked by the depolarizing command subsided to a level no greater than the leak current produced by the preceding hyperpolarizing command. In the presence of Ca^{2+} there is an additional outward current showing appreciable noise. b, Power spectra of current noise (sum of 32 spectra) in another cell recorded at the holding potential (-40 mV) and during the last 64 s of a 2-min depolarizing command to -10 mV in normal Ringer solution (corrected by subtraction of the -40 mV spectrum, although this made negligible difference). The curve is drawn according to the equation $S(f) = S(0)/[1 + (f/f_0)^2]$ where S(f) is the power $S(f) = S(0)/[1 + (f/f_0)^2]$ where S(f) is the power $S(f) = S(0)/[1 + (f/f_0)^2]$ where S(f) is the power at $S(f) = S(f)/[1 + (f/f_0)^2]$ where S(f) is the power at $S(f) = S(f)/[1 + (f/f_0)^2]$ where S(f) is the power at S(f) is 14 Hz. The mean leak-corrected S(f) is the power at S(f) is the power at S(f) is 14 Hz. The mean leak-corrected S(f) is the power at S(f) is

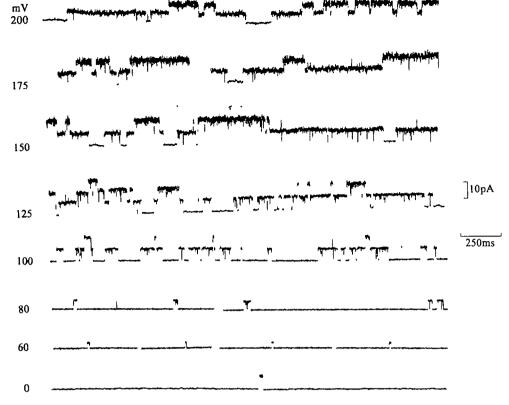


Fig. 4 Single-channel activity in a cultured bullfrog sympathetic ganglion cell. A fire-polished pipette filled with normal Ringer solution (tip resistance 3-5 M(1) was lowered vertically on to the cell membrane and suction gently applied to produce a gigohm seal. The enclosed membrane patch was initially silent but after a few minutes the electrical events illustrated in these records appeared and lasted for ~1 h. The voltages on the left show the potential of the pipette with respect to ground (thus, 0 means that the enclosed membrane patch was at rest potential, a patch potential of 60 means that the membrane patch was depolarized by 60 mV). Recording bandwidth, ~1 kHz

The second point is that, during a depolarizing voltage step following a Ca^{2+} injection, I_C develops exponentially with a time constant at least as short as that of the delayed rectifier current (which is sigmoidal and relatively slow in these neurones²⁶). Because the inward Ca²⁺ current in these cells is also fast $(\tau_{\text{max}} \sim 4 \text{ ms at } 0 \text{ mV})^{11}$, this suggests that I_C might be activated relatively early during a depolarizing event. Figure 2c shows that this is indeed the case, as addition of Ca2+ and/or removal of external Ca2+ slowed outward current development between -20 and +30 mV. Furthermore, in addition to reducing the spike after-hyperpolarization^{4,5}, Cd²⁺ clearly slowed spike repolarization (Fig. 2d). Thus, in at least a proportion of bullfrog sympathetic neurones, the K⁺ current triggered by rapid Ca²⁺ entry may subserve one of the functions more normally fulfilled by the delayed rectifier current.

This work was supported by NIH grants NS 14986 and NS 14920 to P.R.A., an MRC grant to D.A.B., a Wellcome Travel Grant to A.C. and an MDA Fellowship to R.B.C. We thank Claire Adams for culturing the neurones.

Received 14 September 1981, accepted 17 February 1982

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Vasopressin excites hippocampal neurones

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Hypothalmic supraoptic and paraventricular neurones were the first peptidergic cells to be described in the mammalian brain¹. Most of these large nerve cells synthesize either oxytocin or vasopressin2, as well as their respective neurophysins, and project to the posterior lobe of the pituitary gland. This system of neurones is responsible for the release of these peptides into blood vessels of the posterior lobe in response to appropriate stimuli. Data obtained using immunoassay and immunocytochemical methods, however, have revealed vasopressin-like immunoreactive material^{3,4} as well as vasopressin-positive axons⁵⁻⁷ and presynaptic terminals⁵ in other central nervous system (CNS) locations. Moreover, vasopressin has been shown to affect some aspects of animal behaviour, including memory retention⁹⁻¹³. It has therefore been suggested that vasopressin may act as a neurotransmitter or neuromodulator at synapses in the brain. We show here that low concentrations $(10^{-8} -$ 10⁻⁶ M) of vasopressin powerfully and reversibly increase the rate of firing of neurones in the CA1 area of hippocampal slices from rat and that this effect can be fully antagonized by an anti-vasopressor vasopressin analogue. Hippocampal neurones obtained from Brattleboro rats were also excited by exogenous vasopressin.

We prepared transverse slices from the ventral hippocampus of male rats (~250 g body weight), as vasopressin-positive nerve fibres detected by immunocytochemical methods have been shown to terminate on the dendritic tree of pyramidal neurones

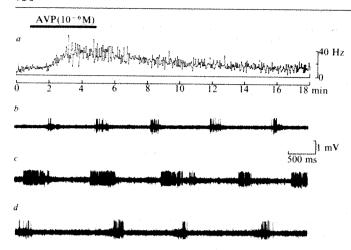


Fig. 1 Effect of vasopressin (AVP, 10^{-6} M) on the rate of firing of a CA1 neurone in a hippocampal slice. a, Ratemeter record; AVP was added to the perfusion medium during the period indicated by the thick bar. b-d, Oscilloscope tracings showing extracellular records (electrode filled with NaCl, 4 M; tip resistance, 5-10 M Ω) of the activity of the same cell before (b), during (c) and after (d) application of AVP.

in the ventral hippocampus^{5,6,14}. Slices (~500 µm thick), were laid on a nylon grid at the interface of a perfusion solution (composition (mM): NaCl 124.0; KCl 5.0; NaHCO₃ 26.0; MgSO₄ 1.4; CaCl₂ 1.0; KH₂PO₄ 1.24; glucose 10.0; temperature 37 °C) and an humidified, oxygenated (95% O2, 5% CO₂) gas mixture. A glass micropipette was lowered under visual guidance into the pyramidal cell layer in the CA1 area of the hippocampus. Substances tested included argininevasopressin (AVP; Bachem or Vega), lysine-vasopressin (LVP; Bachem, Sandoz or Serva), 1-desamino-D-arginine-vasopressin (DDAVP; Ferring), desglycinamide-arginine-vasopressin (DGAVP; Organon), oxytocin (Bachem) and the vasopressor antagonist [1- $(\beta$ -mercapto- β , β -cyclopentamethylpropionic acid)2-(O-methyl)tyrosine]arginine-vasopressin (Peninsula). These compounds were dissolved in the bathing solution immediately before application and perifused at a rate of 2.0 ml min⁻¹. Extracellular recordings were obtained in 43 slices from cells which displayed short intermittent bursts of activity in control solution.

AVP and LVP induced a sustained increase in firing rate in 56 of 63 of these cells and prolonged the duration of the bursts without markedly altering the inter-burst intervals (Fig. 1). The threshold concentration of vasopressin which accelerated cell firing was $\sim 10^{-8}$ M (Fig. 2). Maximal stimulation with AVP occurred at $\sim 10^{-6}$ M and resulted in an increase in mean firing rate from 13.5 ± 1.1 to 44.0 ± 3.2 spikes per s (mean \pm s.e.m., n=47). These concentrations of vasopressin are much lower than those required for the stimulant action of acetylcholine or glutamate ($\sim 10^{-3}$ M), but similar to those at which enkephalin excited hippocampal pyramidal neurones 15.16

The stimulatory effect of vasopressin was slowly reversible and little or no tachyphylaxis was apparent on repeated vasopressin application. We therefore attempted to compare the magnitude of the response with that produced by natural or synthetic analogues. The other mammalian neurohypophysial peptide, oxytocin, which differs from AVP and LVP in amino acid positions 3 and 8 of the molecule, excited hippocampal neurones as effectively as vasopressin in 5 of 6 cells. In contrast, DGAVP, which lacks the glycinamide terminal present in position 9, failed to excite hippocampal neurones (n = 6), even when applied at 10^{-5} M, and glycinamide $(5 \times 10^{-4}$ M) alone had no effect on cell firing (n = 3). DGAVP essentially lacks endocrine activity, but has been reported to exert an effect similar to that of vasopressin on memory and learning 17 .

Brattleboro rats, which are incapable of vasopressin biosynthesis, show behavioural deficits which can be alleviated by injecting vasopressin¹⁸. To determine whether these rats possess

in their CNS the same binding sites for neurohypophysial peptides as normal non-diabetic rats, we compared the effects of exogenous vasopressin on cells in hippocampal slices obtained from Long-Evans rats (n=8) and from homozygous Brattleboro rats (n=7). In the latter, 10^{-7} M vasopressin accelerated neuronal firing and 10^{-6} M did so more powerfully. Doseresponse relationships did not differ significantly between the two strains. As in normal rats, the stimulatory effect of vasopressin was reversibly abolished by the synthetic vasopressin antagonist.

To determine the site of action of vasopressin, small regions (~1 mm² surface area) of the CA1 area were cut from hippocampal slices. Vasopressin was as effective in these trimmed slices as in normal slices. Two further sets of experiments suggested that vasopressin can act postsynaptically. First, vasopressin was applied to slices in which the neurones had been synaptically uncoupled by raising the external Mg²+ concentration to 10 mM. The cells were no longer spontaneously active, but in 4 of 6 cells, they were still excited by 10⁻⁶ M AVP. Second, the stimulatory effect of vasopressin was not reduced by applying antagonists to various suspected transmitters. Thus neither atropine, nor propranolol nor naloxone, interfered with the action of vasopressin.

At least two types of peripheral vasopressin receptor have been recognized. The renal receptor (V2-receptor) is responsible for the antidiuresis induced by vasopressin and is linked to an adenylate cyclase 19,20. The V₁-receptor, which is not coupled to an adenylate cyclase, is present on smooth muscle cells and liver cells21,22 and triggers the vasopressor and glycogenolytic effects of vasopressin on these tissues. The availability of rather selective V2-agonists allowed the testing of the nature of the hippocampal response. DDAVP has virtually no effect on the V₁-receptor but is a powerful antidiuretic compound; in contrast, AVP and LVP exert both effects equally²³. In all four cells examined, DDAVP had a much weaker action on hippocampal cell firing than the same concentration of either AVP or LVP. Further evidence that the effects of vasopressin on hippocampal neurones were unlikely to be mediated by V_2 -like receptors was obtained from application of a V_1 -antagonist²⁴. This compound alone, at 10^{-6} M, had no effect, but

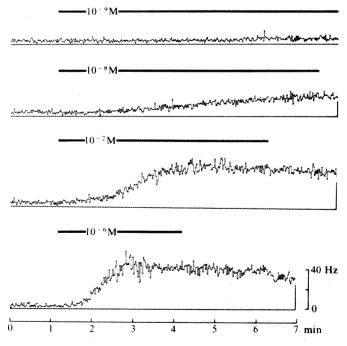


Fig. 2 Segments of ratemeter records illustrating the effects of increasing concentrations of AVP applied to the bath on the rate of firing of a hippocampal neurone, recorded extracellularly. Duration of application (solid black line) and molar concentrations are indicated above each trace.

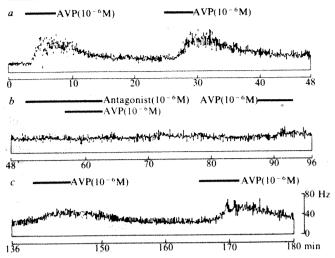


Fig. 3 Effect of the vasopressor antagonistic AVP analogue on the spontaneous and AVP-induced firing of a CA1 hippocampal neurone. Extracellular recording. AVP (10⁻⁶ M), applied at intervals of ~25 min, caused a marked increase in the rate of firing of this cell without showing any evidence of tachyphylaxis (a). The antagonist (10⁻⁶ M) did not affect firing per se, but blocked the effect of AVP for a prolonged period (b); its effect was rapid in onset, but slowly reversible (c). Note that 40 min of record have been deleted between b and c. Peptides were applied during the periods indicated by the thick black lines.

totally and reversibly abolished the effect of vasopressin on cell firing in all five cells tested (Fig. 3).

In invertebrate ganglia, vasopressin (10⁻⁹-10⁻⁷ M) induced or prolonged the burst of pacemaker neurones^{25,26}. In the rat brain, iontophoretically applied vasopressin decreased the rate of firing of cells recorded from the hypothalamic supraoptic nucleus²⁷ and increased neural firing in the locus coeruleus² In the present study, AVP and certain related peptides have been shown to increase the firing rate of hippocampal neurones when added to the medium at concentrations $>10^{-9}$ M. This threshold concentration is at least 50 times higher than the concentration of vasopressin found in cerebrospinal fluid (CSF)²⁹. Even though some peptide degradation by proteolysis may have occurred in the slices and the real concentration at the site where the effects of vasopressin arose is therefore unknown, it seems unlikely that the effect of vasopressin in the hippocampus in situ is mediated by the CSF. We interpret the results as evidence that vasopressin can act as a transmitter or modulator in the mammalian hippocampus. Indeed, other investigators have reported that cells of the CA1 region of the hippocampus are stimulated by several peptides, including enkephalins^{15,16} and somatostatin, vasoactive intestinal peptide, bombesin and CCK-8S (refs 30, 31).

Numerous data have suggested an involvement of vasopressin in facilitation of memory storage and retrieval⁹⁻¹³. DGAVP has similar activity whereas oxytocin has the opposite effect. Here we report a different structure-activity relationship for the action of vasopressin in hippocampal slices: both vasopressin and oxytocin are powerful excitants of neural firing, while comparable concentrations of DGAVP have no effect.

This work was supported in part by grant 3.469.79 from the Swiss NSF.

Received 16 December 1981; accepted 12 March 1982.

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Control of cyclic nucleotide metabolism by non-cholinergic, non-adrenergic nerves in rat thyroid gland

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In addition to the well-established role of thyrotropin (TSH) in controlling metabolism and secretion of thyroid follicular cells 1-3, there is evidence to suggest nervous (cholinergic and mechanisms4-8. control Nerves immunoreactivity to vasoactive intestinal peptide (VIP) have recently been demonstrated in the thyroid gland, while exogenous VIP has been shown to elevate tissue cyclic AMP levels, increase the number of colloid droplets in follicular cells and increase thyroid hormone secretion in vivo. It was therefore suggested that VIP-containing nerves might control thyroid activity9, although a functional non-cholinergic, non-adrenergic innervation of the thyroid has not yet been demonstrated. By adopting the technique of electrical field stimulation previously used on isolated pancreatic segments¹⁰⁻¹³, we have studied cyclic nucleotide metabolism in isolated thyroid fragments and have now demonstrated directly the influence of both cholinergic and adrenergic as well as non-cholinergic, non-adrenergic nerves on thyroid cyclic AMP and cyclic GMP metabolism. In the presence of complete cholinergic and adrenergic blockade, nerve stimulation evoked complex time course changes in tissue cyclic AMP and cyclic GMP levels which could be mimicked precisely by VIP. Electrical stimulation of intrinsic thyroid nerves released, in addition to acetylcholine (ACh) and noradrenaline, a transmitter with the same action on thyroid cyclic nucleotide metabolism as VIP.

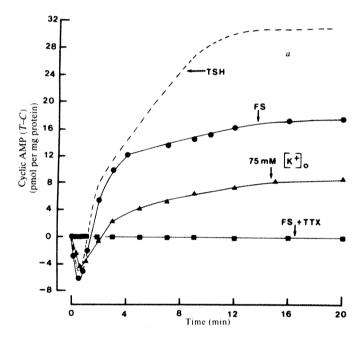
All experiments were performed on isolated fragments of rat thyroid superfused with modified Krebs-Henseleit solution, gassed with 95% O₂, 5% CO₂ and maintained at 37 °C (ref. 10). Mixed fragments (5-10 mg) of the thyroid glands from 16 rats were placed in 8 small Perspex flow chambers (vol 0.6 ml, flow 1 ml min⁻¹). Four of the chambers contained silver electrodes for electrical field stimulation (FS), the remaining four, without electrodes, served as control 'partner' preparations. The preparations (each consisting of the equivalent of two whole thyroid glands) were superfused 30 min before stimulation. At various times following onset of FS or application of drug, both

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Table 1 Effects of field stimulation (FS) in the presence of TTX on tissue cyclic nucleotide levels

Time	Cyclic AMP test (FS+TTX)	Cyclic AMP control	Cyclic GMP test (FS+TTX)	Cyclic GMP control
10 s	9.38	9.46	2.38	2.46
30 s	6.47	6.50	2.25	2.23
45 s	7.31	7.21	3.36	3.41
1 min	5.97	6.15	1.86	1.79
2 min	7.02	7.20	2.71	2.78
3 min	8.10	8.13	2.58	2.67
5 min	8.05	8.25	3.03	3.00
7 min	6.83	6.86	2.48	2.51
9 min	6.70	6.78	2.12	2.11
12 min	9.15	9.17	3.34	3.41
16 min	7.42	7.35	2.69	2.63
20 min	8.31	8.25	2.15	2.25

Field stimulation was continuous at 20 Hz, 50 V, with pulse durations of 1 ms. Mean (\pm s.e.) control levels of cyclic AMP and cyclic GMP were: 7.61 ± 0.29 and 2.60 ± 0.13 pmol per mg protein, respectively (n=12). The mean (\pm s.e.) levels of cyclic AMP and cyclic GMP following FS in the presence of TTX (10^{-6} M) were 7.56 ± 0.31 and 2.58 ± 0.14 pmol per mg protein, respectively (n=12). The data reveal no statistically significant difference between the test and control values (P>0.1).



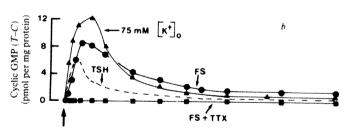


Fig. 1 Time course of changes in the concentration of cyclic AMP (a) and cyclic GMP (b) in superfused rat thyroid segments following electrical field stimulation (FS: 20 Hz frequency, 50 V amplitude, 1 ms pulse width) in the absence (\blacksquare) and presence (\blacksquare) of 10^{-6} M TTX and on exposure of preparations to Krebs solution containing 75 mM K⁺ (\triangle) . Also shown are the cyclic nucleotide concentrations in the presence of 20 mU ml⁻¹ TSH (----). Cyclic AMP and cyclic GMP in control and test frozen tissues were measured at precise time intervals after the start of stimulation using the Radiochemical Centre (Amersham) assay kits TRK 432 and TRK 500, respectively. Total protein was estimated using the Biuret method²¹ and cyclic nucleotide values expressed in pmol per mg protein. Mean $(\pm s,e.)$ control levels of cyclic AMP and cyclic GMP were 7.96 ± 0.18 and 2.38 ± 0.06 pmol per mg protein, respectively (n = 60). Each point on the graph represents the difference in cyclic nucleotide concentration between two similar preparations of thyroid segments, one serving as the test and the other as the control.

test and partner preparations (chamber plus tissue) were frozen in liquid nitrogen. Cyclic AMP and cyclic GMP in control and test frozen tissues were extracted and measured¹³.

The cyclic nucleotide concentration in a particular experimental situation was always expressed as the difference between the concentration in the test and the corresponding control preparation. Each data point in Figs 1-3 therefore represents the difference between two single measurements. The reliability of our method can perhaps best be assessed by inspection of Table 1, which shows cyclic nucleotide values in control and test preparations. Because the test in this case consisted of FS plus tetrodotoxin (TTX), that is, nerve stimulation in the presence of a blocker of nerve action potentials, we have, in effect, two parallel controls. Although there is some time fluctuation, it is clear that this occurs in parallel in the test and control preparations.

Figure 1a shows the time-course changes in tissue cyclic AMP levels during FS in the absence and presence of 10⁻⁶ M TTX and during exposure to 75 mM K⁺. The effect of 20 mU ml⁻¹ TSH is also shown for comparison. An initial, rapid, transient fall in cyclic AMP levels occurs in the first 30 s followed by a slower rise reaching almost steady state by 16-20 min. These changes in cyclic AMP concentration were abolished by superfusion of the preparation with TTX. Figure 1b shows time-course changes in tissue cyclic GMP levels during FS, on exposure to 75 mM K⁺ and during FS in the presence of 10⁻⁶ M TTX. The changes evoked by TSH are also shown. The early, rapid fall in cyclic AMP levels is accompanied by a rather slower, transient increase in cyclic GMP levels, maximal after ~1-2 min, then declining slowly, returning to the control level after 10-12 min, and remaining there throughout the stimula-

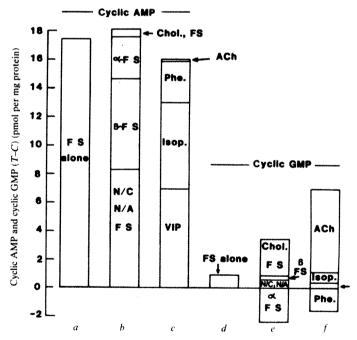


Fig. 2 Histograms showing the effects of electrical field stimulation (FS) and various agonists (for example, Phe., phenylephrine; Isop., isoprenaline; ACh, acetylcholine; and VIP, vasoactive intestinal peptide), in the absence and presence of various combinations of different blockers, on cyclic AMP (a-c) and cyclic GMP (d-f) levels (data from Table 1). a, FS in the absence of blockers. b, N/C, N/A, FS, non-cholinergic and non-adrenergic nervous component (presence of cholinergic and adrenergic blockers); β-FS, β-adrenergic nervous component (presence of cholinergic and α-adrenergic blockers and subtraction of N/C, N/A component); α-FS, α-adrenergic component (presence of cholinergic and β-adrenergic blockers and subtraction of N/C, N/A component); Chol. FS, cholinergic nervous component (presence of α- and β-adrenergic blockers and subtraction of N/C, N/A component). c, VIP (10^{-7} M); Isop. (10^{-5} M); Phe. (10^{-5} M); ACh (10^{-5} M). d, FS in the absence of blockers. e, α-FS, α-adrenergic nervous component; β-FS, β-adrenergic nervous component; β-FS, β-adrenergic nervous component; β-FS, β-adrenergic nervous component. f-FS, g-adrenergic nervous component. g-FS, g-Adrenergic nervous component.

tion period. TTX (10^{-6} M) abolished the cyclic GMP concentration changes evoked by FS.

The series of experiments summarized in Fig. 1 (thyroids from 200 rats, yielding 50 tests and 50 control preparations) demonstrates directly that electrical stimulation of intrinsic nerves causes marked and complex changes in cyclic nucleotide metabolism. In the steady state (after ~15 min stimulation) there is a substantial and sustained elevation in cyclic AMP concentration. As several different transmitters might be involved it was necessary to carry out experiments using various blocking agents. Table 2 shows the results from experiments in which thyroid preparations were superfused for 20 min in the presence or absence of different kinds of stimuli combined with various blockers. The nerve stimulation-evoked increase in tissue cyclic AMP concentration can be explained as the sum of α - and β -adrenergic components in addition to a substantial non-cholinergic, non-adrenergic contribution. The experiments carried out with specific agonist superfusion (isoprenaline, phenylephrine, ACh and VIP) gave results that fit those obtained with the nerve stimulation (Fig. 2). Although there is no unequivocal indication of a cholinergic nervous component in the cyclic AMP results, the results with cyclic GMP make it clear that nerve stimulation releases ACh which acts on muscarinic rather than nicotinic receptor sites (Table 2). In the steady state, nerve stimulation causes only a modest elevation of cyclic GMP concentration because the elevation expected from the cholinergic component is partly balanced by the ability of α -adrenergic stimulation to decrease cyclic GMP levels (Fig. 2).

The finding that the non-cholinergic, non-adrenergic component of the nerve stimulation-evoked increase in cyclic AMP concentration is matched by the effect of VIP (Fig. 2) implicates VIP as the transmitter involved. If this is so, superfusion with VIP should mimic the entire time course of the nerve stimulation (FS)-evoked changes in cyclic AMP and cyclic GMP concentration obtained in the presence of cholinergic and adrenergic blockers. As seen in Fig. 3, this was indeed the case. Figure 3a shows the time-course changes in cyclic AMP levels following FS and exposure to 10^{-7} M VIP and 75 mM K⁺ in the presence of atropine, propranolol and phentolamine. There is a large and rapid increase in cyclic AMP, maximal after 30-60 s, followed by an abrupt, transient fall after 2-3 min. Thereafter, the level of cyclic AMP rises gradually to a secondary increase, reaching a steady-state level after 16-20 min. Figure 3b shows time-course changes in cyclic GMP concentration during application of FS, 75 mM K⁺ and during exposure to 10⁻⁷ M VIP. The early rapid rise and fall in cyclic AMP is accompanied by a slow, transient increase in cyclic GMP, reaching a maximum after 2-3 min before declining towards the control level.

The series of experiments summarized in Fig. 3 (thyroids from 136 rats, yielding 34 test and 34 control preparations)

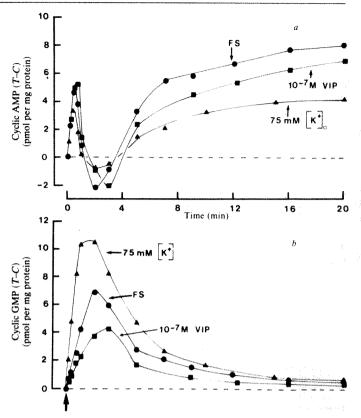


Fig. 3 Time-dependent changes in endogenous cyclic AMP (a) and cyclic GMP (b) levels in rat thyroid segments following FS (\bullet) , during application of 75 mM (\bullet) and (\bullet) and (\bullet) VIP (\bullet) . Atropine $(0.4 \times 10^{-6} \, \text{M})$, phentolamine $(0.4 \times 10^{-6} \, \text{M})$ and propranolol $(0.4 \times 10^{-6} \, \text{M})$ were present in the superfusing solution from the start of the periusion period and throughout the period of stimulation. Cyclic AMP and cyclic GMP concentrations are expressed as in Fig. 1.

demonstrates that the non-cholinergic, non-adrenergic component of the nerve-stimulation response has the opposite initial effect on cyclic AMP levels to that evoked by nerve stimulation in the absence of blockers (Fig. 1). The complex triphasic cyclic AMP response to non-cholinergic, non-adrenergic nerve stimulation is remarkably well reproduced by VIP stimulation. The response is clearly not a non-specific peptide effect because at steady state (20 min), the structurally very different peptide caerulein, used in the same concentration as VIP $(10^{-7} \, \text{M})$, decreases the tissue cyclic AMP concentration (Table 2).

Elevation of extracellular K^+ concentration is generally accepted as a powerful stimulant of neurotransmitter and hormone secretion in a variety of tissues^{13–16}. Figures 1 and 3 demonstrate that high K^+ stimulation does to a large extent mimic the effect of nerve stimulation, although the cyclic GMP

Table 2 Effects of different stimuli (20 min) in the presence or absence of various blocking agents on thyroid cyclic nucleotide levels

			Cyclic AMP			Cyclic GMP	
				(pmol per m	g protein)		
		Test (T)	Control (C)	T-C	Test	Control	T-C
a	FS alone	25,74	8.25	17.49	3.11	2.25	0.86
b		16.45	8.20	8.25	2.84	2.25	0.59
c	FS + atropine + phentolamine	20.77	6.15	14.62	3.17	2.36	0.81
d	FS + phentolamine + propranolol	14.88	6.15	8.73	5.52	2.36	3.16
e	FS + atropine + propranolol	17.36	6.15	11.21	0.55	2.36	-1.81
f	FS + atropine + phentolamine + propranolol + hexamethonium	16.05	7.93	8.12	2.38	2.05	0.33
g	ACh (10 ⁻⁵ M)+phentolamine+propranolol	7.03	6.97	0.06	8.09	2.26	5.83
h	······································	6.92	6.98	-0.06	8.17	2.97	5.20
Ė	ACh (10 ⁻⁵ M) + atropine	6.12	6.05	0.07	2.85	2.65	0.20
į	Phenylephrine (10^{-5} M) + atropine + propranolol	9.62	6.79	2.83	0.79	2.38	-1.59
k	pro	12.98	6.97	6.01	2.94	2.26	0.68
1	VIP (10 ⁻⁷ M) + atropine + phentolamine + propranolol	14.82	7.90	6.92	2.67	2.35	0.32
m	propression anophic promiting	5.21	9.67	-4.46	3.99	2.47	1.52
n		37.24	6.28	30.96	3.00	2.61	0.39

The concentrations of blockers used were (M): atropine, 10^{-6} ; propranolol, 5×10^{-6} ; phentolamine, 10^{-5} ; hexamethonium, 10^{-5} . Each value listed under control and test is the result of a single measurement.

changes are more marked than with electrical stimulation and the effects on cyclic AMP metabolism are weaker. High K+ will, in addition to depolarizing intra-thyroidal nerve endings, depolarize the follicular cell membrane^{17,18}, but this is not accompanied by any change in the membrane resistance¹⁸, as the thyroid follicular cell membrane is electrically inexcitable 17. All effects of FS are abolished by TTX19. As thyroid follicular cells do not fire action potentials^{17,18}, the only specific site for TTX action is on intra-thyroidal nerves. Furthermore, ACh, adrenaline, VIP and TSH have no effect on thyroid follicular cell membrane potential and resistance 17,18

The present work has shown that two recognized methods of stimulating nerves in vitro elicit similar changes in thyroid cyclic nucleotide levels, lending powerful support to the hypothesis that intra-thyroidal nerves can influence thyroid follicular cells directly. The pattern of the innervation is complex. Clearly, further work is required before the neurotransmitters responsible can be definitely identified, but the results presented here do show functional cholinergic, adrenergic as

Received 16 July 1981, accepted 3 March 1982

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well as non-cholinergic, non-adrenergic innervation of the thyroid gland. As exogenous VIP closely mimics the noncholinergic, non-adrenergic nerve response (Fig. 3) and VIPimmunoreactive nerves have been demonstrated in the thyroid9, it seems likely that the neurotransmitter is VIP or a closely related peptide. Nerve-mediated mechanisms in the control of thyroid function could constitute a sensitive and rapidly responding mechanism, allowing accurate modification of glandular metabolism to accommodate fluctuating requirements of the organism.

The finding that all the stimulation procedures resulted in changes in both cyclic AMP and cyclic GMP levels emphasizes the danger of focusing exclusively on cyclic AMP metabolism. The cyclic nucleotide changes generally display a reciprocal pattern which has also been observed in the frog heart 20 and the pancreas¹³. The functional implication of this complex interrelationship obviously deserves much further attention.

This work was supported in part by the MRC. We thank Mr B. Weryk and Mrs K. Kleppang for technical assistance.

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A differentiation factor required for the expression of cytotoxic T-cell function

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Effector cytotoxic T lymphocytes (CTLs) are induced from resting precursors (small lymphocytes) which do not express cytotoxic activity. The process of CTL activation, which occurs in 2-7 days following mitogen or antigen stimulation, thus involves a differentiation event and normally also involves proliferation. These events in CTL activation seem to be induced, at least in part, by soluble, antigen-nonspecific mediators which reach the CTL precursor as a consequence of cell-cell interactions during the immune response. The initial evidence for this was the finding that in addition to CTL precursors, Ia-positive macrophage-like accessory cells1-4, and helper T cells⁵⁻¹⁰ are required for the induction of CTL activity in vitro. Furthermore, the requirement for accessory cells can be replaced with factors present in concanavalin A (Con A)induced cultures of spleen cells11. There is evidence that T-cell products such as interleukin-2 (IL-2, formerly T-cell growth factor)¹¹⁻¹⁵ as well as accessory cell products, such as interleukin-1 (IL-1, formerly lymphocyte activating factor) 4,15 involved in T-cell activation, although the precise roles of these factors in CTL activation are poorly understood. Here we provide evidence for a factor, different from IL-1 and IL-2, which is present in supernatants of Con A-activated mouse spleen cells and is required for the differentiation of CTL precursors into active CTLs.

The experimental system used involved the Con A-mediated polyclonal induction of CTLs from T-cell populations¹⁶. The responding T-cell population was depleted of accessory cells so that Con A-induced proliferation 17 and differentiation were

dependent on exogenous factors. CTLs were assayed after 40 h for cytotoxic activity against 51Cr-labelled P815 targets in the presence of phytohaemagglutinin (PHA); this assay registers the activity of all CTLs, regardless of their antigen specificity^{16,18}. We wished to determine whether IL-2, which is assayed by its capacity to maintain the proliferation of cloned CTLs¹⁹, is sufficient to support the Con A-induced differentiation of CTLs from accessory cell-depleted T-cell populations. In initial experiments we compared two sources of IL-2: supernatants from Con A-activated spleen cell cultures (spleen Con-A Sn)^{12,13} and supernatants from cultures of Con A-activated T-cell hybridoma AOFS 21.10.9 cells (AOFS Con A Sn). AOFS 21.10.9 (provided by Drs J. Kappler and P. Marrack) is a cloned, murine T-cell hybridoma^{20,21} which releases IL-2 on stimulation with Con A or antigen.

Our initial observation was that T cells activated with Con A plus AOFS Con A Sn were induced to proliferate (Fig. 1b), but did not mature into active CTLs after 40 h (Fig. 1a). In contrast, T cells activated with Con A plus spleen Con A Sn were efficiently induced to differentiate into cytotoxic cells

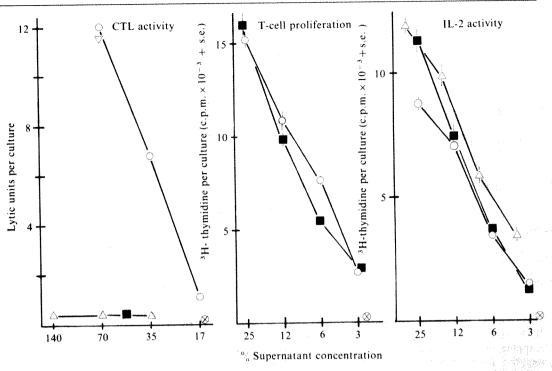
Table 1 Lyt-2-positive T cells proliferate in response to Con A plus AOFS Con A Sn

	³ H-thymidine (c.p per culture inc	
Additions to test culture	T cells	Lyt-2-positive T cells
	0.3 ± 0.1	0.2 ± 0.1
Con A (2 µg ml ⁻¹)	3.4 ± 0.6	1.6 ± 0.2
Con A + 25% spleen Con A Sn	152.4 ± 4.1	357.9 ± 10.4
Con A+12% spleen Con A Sn	108.2 ± 3.2	246 5 ± 11.6
Con A + 25% AOFS Con A Sn	159.1 ± 9.2	336.8 ± 25.6
Con A + 12% AOFS Con A Sn	99.4±32	307 8±27 5

T cells were purified as described in Fig. 1 legend. Lvt-2-positive T cells were purified from accessory cell-depleted T-cell populations by positive selection on plastic dishes³⁰ coated with monoclonal anti-Lyt-2 antibodies (N. Landau and M J B., in preparation). 60×10^6 T cells in 4 ml were incubated for 40 min in the cold on a 100-mm Petri dish which had been coated for 1 h in the cold with 20 µg of purified AD4(15)³¹ anti-Lyt-2 antibodies. The non-adherent cells were removed, the plates were washed six times and the adherent cells (17% of applied cells in this experiment) were removed from the dish by pipetting. T-cell populations selected in this fashion are >95% Lyt-2+. The proliferative response of T cells and Lyt-2+ T cells were determined as in Fig. 1 legend.

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Fig. 1 IL-2 is not sufficient to support the differentiation of CTLs from accessory celldepleted T-cell populations. Spleen Con A Sn^{12,13} was prepared by culturing spleen cells $(7 \times 10^6 \text{ cells ml}^{-1})$ from BALB.K mice with 2 ug ml Con A for 24 h in RPMI-1640 medium containing 5% heat-inactivated fetal calf serum, 50 µM 2-mercaptoethanol, 0.02% 5 mM HEPES, glutamine, 50 µg ml⁻¹ gentamycin, 100 Uml penicillin and 100 µg ml-1 streptomycin (complete medium). AOFS Con A Sn^{20,21} was prepared hv activating AOFS-21.10.9 cells (5) 105 cells ml-1) with µg ml-1 Con A for 24 h in complete medium. The cells were removed by centrifugation and the supernatants sterilized by filtration. Excess Con A was removed from AOFS Con A Sn by passage through a column of Sepharose-4B which had been coupled with p-aminophenyl-a-D-mannopyranoside. centrated AOFS Con A Sn was prepared from AOFS Con A Sn (prepared as above,



except with 0.5% fetal calf serum and 5 μg ml⁻¹ Con A) by precipitation with ammonium sulphate. The fraction that precipitated between 40 and 80% ammonium sulphate, which contains virtually all the IL-2 activity, was resuspended in 1/30 the original volume, and dialysed extensively against RPMI-1640 medium; the concentrations of concentrated AOFS depicted as based on the original volume. Accessory cell-depleted T-cell populations were prepared by passing 5×108 lymph node cells from BALB.K mice through a column containing 3.5 g of washed nylon wool³², and treating the non-adherent cells with ATH anti-ATL serum (anti-Ia* serum) and rabbit complement essentially as described previously³¹. Surviving cells ('T cells') were washed twice before use. The capacity of the supernatants to induce the differentiation of CTLs from accessory cell-depleted T-cell populations was determined by incubating aliquots of 4×10^6 T cells in wells of Costar 3524 plates with $2 \mu g$ ml⁻¹ Con A and the indicated concentration of supernatant in a total volume of 1.5 ml complete medium. After 40 h at 37 °C in an atmosphere of 5% CO₂ in air, the lectin was dissociated from the activated cells by incubating them with α -methyl mannoside (50 mM) for 10 min at room temperature. The cells were washed once, resuspended in 1 ml and serial dilutions of this assayed for cytotoxicity against ⁵¹Cr-labelled P815 targets in the presence of 10 μ g ml⁻¹ PHA-P¹⁶ (Difco) for 6 h essentially as described previously³¹. Lytic units are a relative measure of CTL activity, and are calculated from complete titrations of effector cells versus the per cent specific lysis of target cells. One lytic unit³³ is the number of CTLs which will cause 30% specific lysis of 4×10^4 target cells in this assay period. Comparable numbers of cells were recovered from Con A-activated cultures supplemented with either spleen Con A Sn or AOFS Con A Sn. Con A-dependent proliferation of accessory cell-depleted T-cell populations was determined by inc

Fig. 1a, b). (Cytotoxic cells induced with Con A and spleen Con A Sn were sensitive to complement-mediated lysis with monoclonal anti-Thy-1 or anti-Lhy-2 antibodies, and are thus conventional CTLs (data not shown).) As can be determined from the titration curves shown in Fig. 1c, the two kinds of supermatant had comparable levels of IL-2, and of factors which induce the proliferation of Can A-activated accessory cell-depleted T cells (Fig. 1b). In addition, AOFS Con A Sn did not suppress the induction of CTLs by spleen Con A Sn when included in the same culture (Fig. 1a). These results suggest that a factor(s) other than IL-2, present in spleen Con A Sn but not in AOFS Con A Sn, is required for CTL differentiation. We call this factor 'CTL differentiation factor' (CDF).

Because AOFS Con A Sn induces T-cell proliferation without inducing differentiation into CTLs, it seemed possible that CTL proliferation and differentiation were controlled by separate factors, IL-2 and CDF, respectively. However, although AOFS Con A Sn supports the growth of CTL lines, it was possible that CTL precursors do not proliferate in response to Con A plus AOFS Con A Sn.

To ascertain whether AOFS Con A Sn plus Con A induces the proliferation of Lyt-2-positive T cells (that is, the T-cell subset which contains CTL precursors²²), we purified Lyt-2-positive T cells by absorption to plastic dishes coated with monoclonal anti-Lyt-2 antibodies, and compared the proliferative responses of these cells with the responses of unselected T cells. The proliferative response of Lyt-2-positive T cells to Con A plus AOFS Con A Sn was actually larger than the com-

parable response of unselected T cells (Table 1). In a related experiment, we determined whether Con A plus AOFS Con-A Sn induced the blast transformation of Lyt-2-positive T cells in cultures of unseparated T cells. Density step gradients were used to isolate blast cells (activated cells) from 40-h cultures of accessory cell-depleted T-cell populations activated with Con A plus AOFS Con A Sn. The proportion of these T cells susceptible to lysis with monoclonal anti-Lyt-2.2 antibodies plus complement (27%) did not differ significantly from the proportion of Lyt-2-positive blast cells in cultures activated with Con A plus spleen Con A Sn (32%). Thus, we find no evidence that AOFS Con A Sn is less effective in inducing the proliferation or blast transformation of CTL precursors than is spleen Con A Sn.

In further experiments we determined some of the physicochemical properties of CDF. Spleen Con A Sn was treated in various ways, and then tested for CDF and IL-2 activities. Dialysis against culture medium resulted in a minimal loss of CDF activity, which shows that CDF is a macromolecule (Table 2). Furthermore, treatment of CDF-containing supernatants with trypsin abolished CDF activity, even when the digested sample was supplemented with AOFS Con A Sn for the CDF assay. Thus, CDF has a polypeptide component which is essential for activity. Dialysis of spleen Con A Sn against an acidic (pH 2.0) buffer abolished CDF activity but had no effect on IL-2 activity. Thus, unlike IL-2²³, CDF is acid sensitive.

The finding that CDF activity is abolished by digestion with trypsin is one piece of evidence that CDF is not identical to

Table 2 Physicochemical properties of CDF

Expt	Treatment of Sn	indu	CDF activity secific lysis by seed with indi- entration of te	CTLs cated	³ H-thymidi clone c	ated	
		50%	25%	12%	25%	12%	6%
1	Untreated	32	19	6	8.004 ± 58	6.244 ± 261	3.715 ± 203
	Dialysis vs. medium	21	12	4	10.802 ± 397	7.091 ± 400	-,
	Dialysis vs. pH 2	2	2		8.598 ± 96	7.051 ± 400 7.257 ± 44	$3,805 \pm 312$
2	Untreated	46	27	8	8.929 ± 299	$7,237 \pm 44$ 8.016 ± 234	$3,509 \pm 750$
	Dialysis vs. medium	22	18	6	8,218 ± 452	.,	7.118 ± 795
3	Untreated	29	25	U		$7,205 \pm 181$	$6,674 \pm 422$
	Trypsin treated	3	23		11,743±795 297±83	$10,278 \pm 719$	$5,719 \pm 313$
	Trypsin treated, restored with AOFS Sn	6	2		29/±83	469 ± 81	All Annual Control
	Control for trypsin treatment	39	23		10 210 : 114	0.400 #24	minute.
	AOFS Sn	39	43	6	$10,319 \pm 116$	$9,123 \pm 722$	$5,141 \pm 478$

For expts 1 and 2, 10-ml samples of spleen Con A Sn were either dialysed against two changes (250 ml each) of complete medium (see Fig. 1 legend) or dialysed against 0.1 M glycine, 0.15 M NaCl, pH 2.0, for 18 h, followed by dialysis against two changes (250 ml each) of complete medium. For expt 3, we used serum-free spleen Con A Sn prepared as described in Fig. 1 legend, except that the medium was supplemented with 10 µg ml⁻¹ of bovine serum albumin instead of serum. Samples of serum-free spleen Con A Sn were treated with 20 µg ml⁻¹ trypsin (TPCK treated, Millipore) for 1.5 h at 37 °C, at which time fetal calf serum (which contains trypsin inhibitors) was added to a final concentration of 5%. As a control (expt 3, 4th line) a sample of serum-free spleen Con A Sn was incubated in identical conditions, except that trypsin was added only after the addition of serum. In a separate experiment, treatment of supernatants with Difco trypsin also abolished CDF and IL-2 activities (not shown). Treated supernatants were sterilized by filtration and tested for CDF and IL-2 activities as described in Fig. 1 legend, except that cytotoxic activity is depicted in terms of the % specific lysis of target cells at a single effector to target ratio (25:1). In one case (expt 3, 3rd line), the test supernatant was supplemented with concentrated AOFS Con A Sn (to an adjusted concentration of 140%) for the CDF assay.

IL-1, which is trypsin insensitive²⁴. In addition, we have tested IL-1 preparations from two sources (Dr S. Mizel, and Drs J. Kappler and P. Marrack) in our assay: both preparations were negative for CDF activity, even if supplemented with excess AOFS Con A Sn before the test (data not shown). Taken together, our results suggest that CDF activity resides in a molecular species that is distinct from IL-1 and IL-2.

To demonstrate further the molecular independence of IL-2 and CDF, we attempted to remove IL-2 selectively from spleen Con A Sn in such a way that the supernatant retained CDF activity. IL-2 was depleted from spleen Con A Sn by absorption with cells of an IL-2-dependent cloned CTL line, CTLL-15H (ref. 25). Following absorption, the supernatants were dialysed and tested for CDF and IL-2 activities. Although virtually all IL-2 was removed (Fig. 2b), the absorbed supernatants induced the differentiation of CTLs with ~50% the efficiency of unabsorbed spleen Con A Sn (Fig. 2a). A mixture of the absorbed spleen Con A Sn and AOFS Con A Sn was as effective as unabsorbed spleen Con A Sn in inducing CTL activity (Fig. 2a). From these data, we conclude that CDF is separable from IL-2;

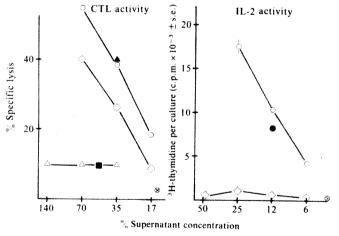


Fig. 2 Spleen Con A Sn, depleted of IL-2, induces CTL differentiation. IL-2 was depleted from spleen Con ASn by absorption with $2\times10^6\,\mathrm{ml}^{-1}\,\mathrm{CTLL}\text{-}15\mathrm{H}^{25}$ (IL-2-dependent CTL line) cells for 24 h at 37 °C. Cells were removed by centrifugation and the supernatant was dialysed against two changes of 250 ml complete medium and sterilized by filtration. T cells were prepared and assays were performed as for Fig. 1, except that instead of lytic units CTL activity is expressed as the % specific lysis at an effector to target ratio of 25:1. Supernatants tested were spleen Con A Sn (○), IL-2-depleted spleen Con A Sn (♦), AOFS Con A Sn (■), AOFS Con A Sn concentrated by ammonium sulphate precipitation (% Sn based on original volume) (A), a mixture of 35% IL-2-depleted Con A Sn and 30% concentrated AOFS Sn (▲), a mixture of 12% spleen Con A Sn and 25% IL-2 depleted spleen Con A Sn (●), and no supernatant (⊗).

furthermore, because the absorbed supernatant had no measurable IL-2 activity and did not inhibit the IL-2 assay significantly (Fig. 2b), it is unlikely that CDF is simply a modified form of IL-2 with both IL-2 and CDF activities.

It is interesting that we find no requirement for exogenous IL-2 in this assay system (Fig. 2a), particularly as IL-2 is implicated in most models of CTL differentiation $^{6.7,11-14}$. In fact, our data do not rule out the involvement of IL-2 in CTL differentiation, because it is possible that Con A-activated T cells in the test culture produce IL-2 in response to factors in the IL-2-depleted spleen Con A Sn.

The absence of a requirement for factors other than IL-2 in CTL induction in other published reports may be because in such studies, spleen Con A Sn was the sole source of IL-2, and biochemical separation of IL-2 from other factors in spleen Con A Sn is often difficult. In addition, we have found that with extended culture periods (4-5 days) CTL activity appears in cultures to which no exogenous CDF was added (data not shown). This may be due to in situ production of CDF by cells in the test culture.

We do not know whether CDF is required to maintain the CTL activity of mature CTLs. The factor does not seem to bind avidly to cloned, mature CTLs, because in conditions in which IL-2 is depleted from spleen Con A Sn by absorption to IL-2-dependent, cloned CTLs, CDF activity is not depleted (Fig. 2).

Recent studies of soluble factors involved in the antibody response have provided evidence for separate factors which regulate the growth and differentiation to immunoglobulin secretion of anti-immunoglobulin or antigen-activated B cells²⁶⁻²⁹. Our data suggest that CTL activation may also involve growth-inducing and separate differentiation-inducing activities-IL-2 and CDF, respectively. Further work will be required to determine the cellular source and specific mechanism of action of CDF.

This work was supported by USPHS grant AI-14269. We thank Lorraine Gemmell for technical assistance, Fern Brown for typing the manuscript, and Dr David Parker for performing the absorption of Sp Con A Sn with CTLL cells.

Received 24 September 1981; accepted 22 February 1982.

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Interferon inhibits the suppressor T cell response of delayed-type hypersensitivity

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We have recently shown that pretreatment of BALB/c mice with Corynebacterium parvum preferentially inhibits suppressor T lymphocytes (T_s) which have been induced by an epicutaneous antigen overload or by intravenous (i.v.) injection of 2,4dinitrobenzenesulphonic acid (DNBSO₃)². Serum obtained 24 h after C. parvum injection had a similar suppressive effect on T₈ but no effect on T effector lymphocytes of delayed-type hypersensitivity $(T_{DH})^3$. Inhibition of T_8 by this serum could be neutralized by an anti-interferon globulin4. A crude mouse fibroblast interferon (IFN) preparation also inhibited the T_s response4, which suggested that IFN in C. parvum serum might be the Ts inhibitory factor. We have now studied the effect of an electrophoretically purified mouse interferon (IFN-a and β) preparation on the T_s response to dinitrofluorobenzene (DNFB) in BALB/c mice. Small amounts (1,000 U) of the preparation inhibited the generation of T₅ by DNBSO₃ in vivo, and the function of T_s both in the recipient animals when injected i.v. 2 h after transfer of the Ts and after incubation of the spleen cells from tolerant animals with interferon before transfer into recipients. The TDH response was not affected by this amount of interferon.

BALB/c mice were sensitized to DNFB by the method of Phanuphak et al. $^{1.5}$. Briefly, 15 μ l of 0.5% DNFB were painted on to the shaved abdominal skin of BALB/c mice on two consecutive days; 5 days later one ear was challenged with 0.3% DNFB. Ear swelling was measured 24 h later using an engineer's micrometer and expressed as 10⁻² mm difference in thickness between the unchallenged and challenged ears. Increasing the sensitizing dose of DNFB (50 μ l 0.5%) resulted in diminished contact sensitization¹. Sy et al.⁶ have shown that the diminished immune response is due to the generation of T_s by the antigen overload.

Interferon was prepared in mouse C-243 cells by induction with Newcastle disease virus. It was purified by sequential chromatography on Sepharose-bound poly(U) and anti-interferon globulin as previously described. Electrophoretic analysis on polyacrylamide gel, followed by staining with Coomassie brilliant blue, revealed only the three interferon bands, corresponding to molecular weights of 35,000, 28,000 and 22,000, and no contaminating bands. The 35,000- and 28,000molecular weight species correspond to mouse IFN- β and the 22,000 species to mouse IFN- α (ref. 8). The purified interferon was stabilized with 0.5% BALB/c serum.

Intravenous injection of 1,000 U (Fig. 1c) or 100 U (Fig. 1d) interferon 2 h after the first sensitization with an antigen overload (50 µl DNFB) increased ear swelling compared with untreated or buffer-treated controls. Ear swelling was not diminished in animals treated with interferon 2 h after optimal sensitization (Fig. 1f-j), which shows that TDH cells were not affected by the amount of interferon used. The enhancement of T_s-mediated suppression of contact sensitivity by interferon after antigen overload suggests that interferon inhibits the manifestation of the T_s response as discussed previously¹

In the following experiments (Figs 2-4) T₈ were induced by i.v. injection of 15 mg DNBSO₃ per mouse^{2,5,9}. To confirm the presence of T_s, spleen cells (1×10° cells per mouse) prepared 6 days after DNBSO₃ injection from donor spleens were transferred to naive recipients. They were sensitized on two consecutive days with an optimal amount of DNFB and challenged 5 days later. Intravenous injection of 1,000 U interferon 2 h after i.v. injection of DNBSO₃ inhibited the generation of T₅ (Fig. 2d); thus no suppression could be transferred with the spleen cells of interferon-treated animals to the recipients as compared with the untreated (Fig. 2b) or buffer-treated (Fig. 2c) controls. One hundred units of interferon (Fig. 2e) were marginally effective, ten units (Fig. 2f) had no effect. In the following experiment the effect of interferon on Ts in the recipient animals was investigated by i.v. injection of the recipients with interferon 2 h after T_s transfer (Fig. 3). The activity of the transferred Ts was completely abolished by treating the recipient animals with 1,000 U interferon (Fig. 3d). Inhibition of T_s activity has also been observed in recipient animals injected with C. parvum or serum obtained from C. parvum-treated animals^{2,3}

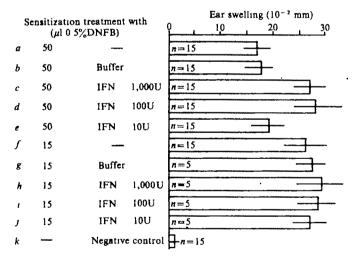


Fig. 1 Effect of interferon on contact sensitivity in animals sensitized with an optimal amount of DNFB (15 µl) and by epicutaneous antigen overload (50 µl). BALB/c mice, 10-12 weeks old, were sensitized with 15 µl (optimal) or 50 µl 0.5% DNFB by painting the shaved abdominal skin on two consecutive days. Interferon or buffer (0.5 ml per mouse) was injected i.v. once 2 h after the first sensitization. On day 5, ear challenge was performed by painting the right ear with 20 µl 0.3% DNFB; 24 h later ear thickness was measured and ear swelling was calculated as the difference in ear thickness between the unchallenged (left) and challenged (right) ear. The results of three experiments are presented in this figure and in Figs 2-4 as mean ear swelling ± s.d All data were analysed by one-way analysis of variance followed by Scheffe's test for least significant difference. Statistical analysis. group a not different from b; group c increased over a and b, group d increased over a and b; group e not different from a and b; group f not different from g, h, k, j; group k (nonsensitized animals, ear challenged) less than all other groups.

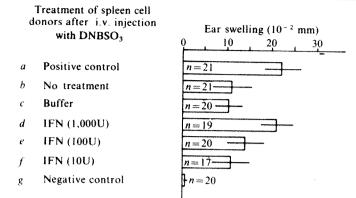


Fig. 2 Effect of interferon on the T_S response in the spleen cell donor treated with DNBSO₃ i.v. Mice treated with DNBSO₃ i.v. (15 mg per mouse) to induce T_S were injected i.v. with interferon or buffer 2 h later. After 6 days spleen cell suspensions were prepared and transferred by i.v. injection to naive recipients (1×10⁸ cells per mouse). The recipients were sensitized with 15 μl 0.5% DNFB and challenged as described in Fig. 1 legend. The results are expressed as mean±s.d. from 2-3 experiments. a, Optimally sensitized animals. Groups b-f, ear swelling in recipients receiving spleen cells from DNBSO₃-treated donors: b, no further treatment of donors; c, donors injected i.v. with 0.5 ml buffer; d-f, donors injected i.v. with 0.5 ml interferon (1,000, 100, 10 U). g, Unsensitized, ear-challenged control. Statistical analysis: group b less than a; c less than a; c not different from b; d not different from a; d increased over b and c; e, f not different from b, c.

Figure 4 shows the results of experiments in which spleen cells from tolerized animals were treated in vitro with interferon before transfer to the recipient animals. The spleen cells were prepared from animals treated with DNBSO₃ 6 days earlier and 1×10° per ml were incubated with 10,000 U interferon per ml for 2 h at 37 °C, or were incubated similarly in the control buffer or transferred immediately after preparation to the recipient animals. After incubation the cells were washed three times in Dulbecco's minimal essential medium and 1×10^8 cells per mouse injected i.v. into the recipient animals. The incubation and washing procedure of the buffer-treated spleen cells did not affect the suppressive capacity of these cells in the recipient animals (Fig. 4c) compared with the non-incubated spleen cells (Fig. 4b). Incubation of the spleen cells with interferon, however, completely abolished their capacity to transfer suppression (Fig. 4d).

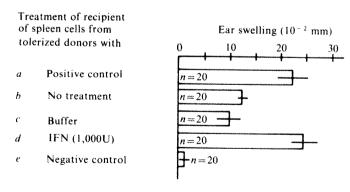


Fig. 3 Effect of interferon treatment of the recipients of T_S on the T_S response. Groups of mice were tolerized as described in Fig. 2 legend. Spleen cell suspensions were prepared after 6 days and transferred to recipients $(1\times10^8~{\rm cells}~{\rm per}~{\rm mouse})$ which were treated with buffer or interferon by i.v. injection 2 h after cell transfer. Sensitization and challenge of the recipients was performed as described in Fig. 1 legend. a, Optimally sensitized animals; b-d, ear swelling in recipients treated 2 h after T_S cell transfer with: b, no treatment; c, buffer; d, interferon. Statistical analysis from two to three experiments: group b less than a; c less than a; c not different from b; d increased over b and c; d not different from a.

The results reported here show that T_s of delayed-type hypersensitivity are inhibited by electrophoretically pure interferon; the in vitro treatment of Ts with interferon revealed a subpopulation of immune regulatory lymphocytes highly sensitive to interferon (1 U of interferon inhibits T_s cells present in 10^s spleen lymphocytes). The T_{DH} response is not affected by the amount of interferon which inhibits T_s. Inhibition of T_{DH} probably requires much higher amounts as suggested by the inhibitory effects of interferon on expression of delayed-type hypersensitivity to sheep red blood cells and picryl chloride 10. Interferons have been shown to affect the immune reaction in vitro and in vivo (for review see ref. 11). Interferon augments the cytotoxic activity of natural killer cells¹² and inhibits cell-mediated immune responses in vivo¹⁰ and in vitro¹³. Its effects on cell-mediated immunity, as measured in experimental contact allergy induced by picryl chloride or in delayed reaction to sheep red blood cells¹⁰, strictly depends on the interferon dose used and the timing of injection. Whereas application of interferon before sensitization results in a decreased immune

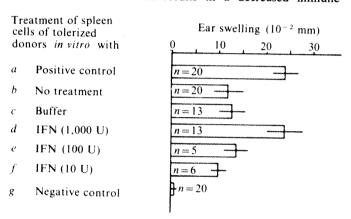


Fig. 4 Treatment of spleen cells from tolerized donors with interferon in vitro before transfer. Spleen cell suspensions were prepared from tolerized donors as described in Fig. 2 legend; 1×10^9 cells were incubated at 37 °C for 2 h, 7% CO₂ in 1.0 ml Dulbecco's minimal essential medium containing control buffer or interferon. After incubation the cells were washed three times with the medium and injected i.v. into recipients $(1 \times 10^8 \text{ cells per mouse})$. Recipients were sensitized and challenged as described in Fig. 1 legend. a, Optimally sensitized animals; b-f, ear swelling in animals receiving spleen cells treated in vitro with: b, no treatment; c, buffer; d-f, interferon $(1,000, 100, 10 \text{ U per } 1 \times 10^8 \text{ spleen cells})$. Statistical analysis of one to two experiments: groups b, c less than a; c not different from b; d increased over c, b; d not different from a; e, f not different from b, c.

response, injection after sensitization increases the immune response 14. In our system which is able to differentiate between T_{DH} and T_S, small amounts of interferon were capable of increasing the immune responsiveness by selectively suppressing T_s. This probably provides an important clue to the observed enhancing effect of IFN. At the moment it is not clear which T_s (suppressing the afferent or efferent limb of the immune response) may be preferentially inhibited by interferon. The mechanism of interferon-mediated inhibition of T_s may be explained by the suppressive effect on lymphocyte proliferation which has been observed in several in vitro systems 15. Inhibition of T_s proliferation would explain the inhibitory effect of interferon in the donor, the recipient and the in vitro treatment of Ts if one assumes that further clonal expansion is required for the T_s function in the recipient animals. An alternative explanation would be inhibition of effector function of T_s by interferon or an indirect action on T_s by contra suppressor cells¹⁶

The results obtained with the purified interferon preparation confirm our previous findings that T_s inhibition by $C.\ parvum^2$ and serum obtained from $C.\ parvum$ -treated animals³ is mediated by interferon⁴. Although it is widely accepted that interferon is immunosuppressive in vitro and in vivo, recent

observations17 showing that interferon enhanced the specific antibody response to sheep red blood cells in vivo and our present results suggest a stimulatory effect of interferon. The interference of interferon with Ts may augment immune responses and be an important protective mechanism against viral and bacterial infections.

We thank Maria-Theres Keuck and Reinhild Luster-Haggeney for technical assistance and Brunhilde Scheibel for typing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (grant KN 120/4).

Received 27 November 1981: accepted 22 February 1982

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Primary structural studies of the Oa-2 alloantigen: implications for the evolution of the MHC

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The murine major histocompatibility complex (MHC) contains a number of loci encoding cell-surface glycoproteins of molecular weight (MW) 40,000-45,000 which are noncovalently associated with β_2 -microglobulin. The products of this large multigene family, collectively referred to as class I gene products, include H-2K,-D,-L and -R, which are encoded by the H-2 complex, and Qa-1, -2 and TL, encoded by the Tla region telomeric to H-2 (refs 1-5). Functional studies have shown that the H-2K, -D and -L gene products serve as important self-recognition structures in immune responses^{6,7}; a functional role for the other class I gene products has not been established. Tryptic peptide map comparisons and primary sequence analyses have indicated that H-2K, -D and -L are 70-85% homologous with respect to their amino acid sequences⁸⁻¹⁰. The data, however, have failed to reveal structural features unique to gene products of a given locus. Recent tryptic peptide map comparisons of the Tla gene products, Qa-2 and TL, have shown that Qa-2, H-2K and -D are more closely related to each other than to TL^{11,12}. Here we present the NH2-terminal sequence of the Qa-2 alloantigen. Our results, together with those of previous studies, show that Qa-2 has significant homology to H-2 antigens but differs in that Qa-2 molecules lack extensive polymorphism and the Qa-2 heavy chain has two additional NH2-terminal amino acids and several critical amino acid interchanges compared with H-2 antigens.

conventional antiserum to Qa-2 alloantigens $(B6.K1\alpha B6)$ is potentially polyvalent with possible reactivities to Qa-2, -3, -4 and -5 (ref. 13). However, the immunoprecipitating activity of the K1aB6 serum is directed against the Qa-2 alloantigen as absorption of this serum with thymus (Qa--2+3-4-5-) or EL-4 lymphoma (Qa-2+3-4+5-) cells removes

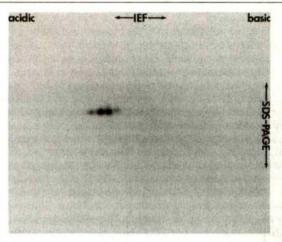


Fig. 1 Two-dimensional gel electrophoresis of the Qa-2 alloantigen. Spleen cells (5×10^7) from C57BL6/J (B6) mice were radiolabelled by lactoperoxidase-catalysed iodination31 labelled cells were washed with phosphate-buffered saline (PBS) and lysed in PBS containing 0.5% NP40 at 4 °C for 15 min. Nuclei and debris were removed by centrifugation and the cytoplasmic extract dialysed at 4 °C against PBS for 16 h. The dialysed extract was centrifuged at 10,000 g for 15 min to remove insoluble material before immunoprecipitation. Immunoglobulin was precleared from the lysate by incubation with rabbit anti-mouse immunoglobulin (RαMIg, 200 μl per 5×107 cells) at 4°C for 45 min, then protein A-bearing Cowan I strain of Staphylococcus aureus was added and the mixture incubated at 4 °C for 45 min. Immunoglobulin-depleted lysates were then treated with normal mouse serum (200 µl per 5×10⁷ cells, 4 °C, 45 min), followed by two additions of Cowan I strain of S. aureus. The precleared lysate was then reacted with anti-Qa-2 serum at 4°C for 45-60 min. Immune complexes were removed by adding goat anti-mouse immunoglobulin (Ga MIg). The immunoprecipitates were washed three times with PBS containing 0.1% SDS, 0.25% NP40 and 0.2% deoxycholate followed by one wash with PBS. The washed immunoprecipitate was then analysed by two-dimensional gel electrophoresis as described by O'Farrell³² and modified by . Gels were exposed to Fuji Rx X-ray film using a Dupont Cronex Lightning-plus intensifying screen. Exposure was for 1-2 weeks at -70 °C. IEF, immunoelectrophoresis; SDS-PAGE, SDSpolyacrylamide gel electrophoresis.

all immunoprecipitating activity14. Since it was critical for sequence studies to establish that this antiserum precipitates a single species of 40,000-MW heavy chain, Qa-2 antigens were immunoprecipitated from lysates of radioiodinated C57BL/6 spleen cells and the immunoprecipitates were analysed by twodimensional gel electrophoresis. As shown in Fig. 1, the anti-Qa-2 antiserum precipitates several acidic 40,000-MW species. The observed microheterogeneity in Qa-2 is typical of cellsurface glycoproteins varying only in the sialic acid content of their carbohydrate moieties. Consistent with this interpretation is the observation that pretreatment with neuraminidase results in a shift to more basic species (data not shown). These results suggest that a single polypeptide chain carries the serologically defined Qa-2 determinant.

H-2 gene products have extensive polymorphism, for example, 56 alleles at the H-2K locus and 45 at the H-2D locus 15. As our peptide mapping studies indicated that the Qa-2 molecules show extensive homology with H-2 gene products, it was important to determine whether Qa-2 exhibited strainrelated polymorphism. So far, two Qa-2 alleles have been defined by the ability of standard antisera (B6.K1\alpha B6) and complement or cytotoxic effector cells either to kill (Qa-2a) or not kill $(Qa-2^b)$ spleen cells from various inbred mouse strains 13,16 . Both the serological and cellular approaches have failed to detect polymorphism associated with the Qa-2^a allele. It is not clear whether the Qa-2b allele is a true 'null' allele or whether the results are due to a reagent limitation. A failure to detect polymorphism by immunological techniques does not indicate the absence of primary structural polymorphism. Thus

the gene products of the Ss/Slp or H-2L loci show no serologically detectable polymorphism, yet biochemical analysis of the isolated molecules detects structural variations^{17,18}.

We immunoprecipitated Qa-2 alloantigens from various inbred strains bearing the Qa-2a allele and compared their primary structures by tryptic peptide mapping. The sensitivity of this technique in detecting a single amino acid change in a given molecule has been well documented¹⁹. Figure 2 shows that the arginine-labelled tryptic peptide maps of Qa-2 molecules isolated from three different inbred strains (A/J, BALB/c and the wild congeneic B10.KPB-128) are identical. We have examined several additional Qa-2a inbred strains including B10, B6, DBA/1, SWR, DBA/2 and the wild congeneic B10.KEA5; the Qa-2 molecules isolated from these strains also have identical tryptic peptide maps. Note that the background strain used to construct the wild congeneic strains (B10.BR) carries the Qa-2^b allele^{20,21}. Thus, in those wild congeneic strains typed Qa-2^a, the Qa-2 locus probably derived from the wild mouse population. When the allelic products of the H-2K and -D loci were examined using similar methods, only 20-50% of their tryptic peptides were identical⁸ observations indicate that the gene products of the Qa-2 locus do not exhibit the extensive allelic structural variation typical of the most well characterized members (that is, H-2K, -D and -L) of this multigene family. Limited polymorphism may be a property of Tla gene products as serological studies have distinguished two alleles for the Qa-1, -3, -4 and -5 loci and five alleles for the Tla locus¹³. Thus, the present and previous peptide mapping studies present a paradox; Qa-2 is strikingly homologous with H-2 antigens, but is the first member of the class I (or class II) MHC antigens that is not highly polymorphic.

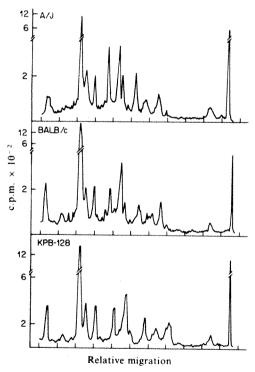


Fig. 2 Tryptic peptide maps of Qa-2 isolated from several inbred mouse strains. Qa-2 molecules metabolically labelled with 3 H-arginine were isolated by immunoprecipitation and SDS-PAGE from a lysate of concanavalin A (Con A)-stimulated spleen cells 11 . The isolated molecules were digested with trypsin and the resulting peptides analysed by cation-exchange chromatography on Technicon chromobeads type P^{11} . Before chromatography, the sample was mixed with 14 C-arginine-labelled P3 myeloma κ chains, which had been digested with trypsin. These peptides served as internal markers facilitating the comparison of each peptide map. The figure shows the arginine-labelled tryptic peptide maps of Qa-2 isolated from A/J (top), BALB/c (middle) and B10.KPB-128 (bottom).

				5					10									
GLY	PR	HIS	SER	LEU	ARG	TYR	PHE	VAL	THR	ALA	VAL	SER	ARG	PRO	GLY	LEU	GLY	GLI
		HIS				LEU		TYR	PHE				VAL		ARG			LEI
				25					30					35				
ARG	TY	MET	GLU	VAL	GLY	TYR	VAL	ASP	ASP	THY	GLU	PHE	VAL	ARG	PHE	ASP	SER	AS
			PHE						VAL							ARG		
				5					10					W. 0-				
GLY	PRO	HIS	SER	LEU	ARG	TYR	PHE	VAL	THR	ALA	VAL	SER	ARG	PRO	SLY	LEU	GLY	GL
		HIS		LEU	*	TYR	PHE	٠			VAL		ARG			LEU		
				MET				SLU		~							-GIH	
********	SER									*******						PHE		
				25					30					35				
ARG	TYR	MET	GLB	VAL	6LY	TYR	VAL	ASP	ASP	THR	GLU	PHE	VAL	ARG	PHE	ASP	SER	ASI
	PHE													AR5				
	PHE	ILE	ALA		********						GLI	**************************************						·
			SER															

Fig. 3 Partial NH₂-terminal sequence of Qa-2 compared with other class I molecules. Qa-2 molecules metabolically labelled with a single amino acid were immunoprecipitated from extracts prepared from Con A-stimulated B6 spleen cells. The immunoprecipitates were denatured, reduced and the 40,000-MW Qa-2 molecule isolated by SDS-PAGE¹¹. The isolated Qa-2 molecules were lyophilized, re-dissolved in H₂O with 1 mg human γ-globulin as carrier, and dialysed against H₂O to remove free SDS. The dialysed Qa-2 molecule was loaded on to a Beckman 890C sequencer for analysis^{22,23}. In each sequencing run, a ³⁵Smethionine-labelled light chain was used as an internal standard. The methionine residues at positions 4, 11 and 13 allowed the determination of a repetitive yield. Thiazolinone derivatives were evaporated under nitrogen and counted in a liquid scintillation counter. Assignments were based on: (1) the appearance of a peak of radioactivity in a given sequencing step; (2) the ability of multiple peaks to fit a standard repetitive yield curve as determined for each run using the ³⁵S-methionine immunoglobulin light-chain standard. The H-2 sequences were obtained from Coligan et al. 1 The upper panel shows the non-aligned H-2Kb and Qa-2 assignments. The lower panel shows the Qa-2 assignments aligned with several H-2 sequences after the introduction of a 2-amino acid gap in the Qa-2 sequence after position 3. Asterisks in the lower panel denote the positions of valine and arginine residues in H-2 molecules, and their counterparts in other species, which are absent from the Qa-2 molecule. The solid lines indicate residues which are identical to those in H-2K

To elucidate the primary structure of the Qa-2 alloantigen, we analysed the NH₂-terminal sequence. Qa-2 alloantigen labelled with a single ³H-amino acid was isolated from lysates of mitogen-activated B6 splenocytes which have been shown to be particularly enriched for the expression and synthesis of Qa-2 (ref. 11). The 40,000-MW heavy chain of Qa-2 was prepared by electrophoresis of the immunoprecipitate and used for NH₂-terminal sequencing^{22,23}. Figure 3 (top) shows the partial NH₂-terminal sequence of Qa-2 in which we have determined the Arg, Leu, His, Phe, Val and Tyr residues within the first 40 amino acids. The sequence of the H-2Kb molecule is also shown. Surprisingly, only 1 of 15 assignments show identity. However, if we assume that during evolution two additional amino acids have been inserted between positions 3 and 7, then shifting the Qa-2 assignments two positions towards the NH₂terminus gives 13/15 assignments which are identical with their H-2K^b counterparts (Fig. 3, bottom). This level of homology confirms the primary structural homology predicted from tryptic peptide map comparisons of Qa-2 and H-2K or -D molecules 11. Further interesting features are: (1) the location of two additional amino acids of Qa-2 on the C-terminal side of position 3 and on the NH2-terminal side of position 7; (2) primary sequence analysis of murine H-2K, -D and -L and their counterparts in other species has shown that the arginines at positions 5, 14, 21 and 35 are highly conserved residues²⁴. Qa-2, on the other hand, has been assigned arginines only at positions 14 and 35 (hereafter we shall refer to the Qa-2 assignments obtained after the 2-amino acid shift). Furthermore, H-2

homologues also show a highly conserved glycine at position 26 (ref. 24); Qa-2 has been assigned a leucine at that position. In addition, Qa-2 lacks a valine at position 9 that is found in H-2Kb, -Kd and -Db molecules. Comparison of the 18 residues (12 assignments, the absence of a valine residue at position 9, and the absence of two arginine residues at 5 and 21) indicates 72-82% homology between Qa-2 and H-2Kb, -Kd, -Db or -Dd. H-2K and -D molecules show 83-94% homology for these 18 positions. These observations indicate that Qa-2 lacks several highly conserved primary structural features of MHCcontrolled major transplantation antigens. Moreover, the Qa-2 alloantigen is rather less related to H-2 alloantigens than they are to each other.

The data therefore suggest that Qa-2 is part of a subfamily of class I gene products, the members of which are distinct at the primary structural and, perhaps, functional levels from members of a second subfamily which includes H-2K, -D and -L. This hypothesis is consistent with the difference between Oa-2 and H-2 antigens with regard to tissue distribution, ontogeny, role as self-recognition units and particularly, extent of polymorphism¹³. Several investigators have postulated that the evolution of polymorphism at H-2 loci was critical for the survival of species/populations against infectious agents^{15,25} The failure of the Qa-2 locus to display extensive polymorphism may indicate a different function for the Qa-2 molecule. Interestingly, tryptic peptide map comparisons of TL with H-2K, -D and Qa-2 indicate that TL is only distantly related to these molecules, which suggests that there may be a third subfamily containing TL (refs 12, 26 and M.J.S., K. McIntyre, J.W.U. and E.S.V., in preparation).

The multigene family encoding class I gene products possibly evolved from a primordial gene that has undergone duplication and subsequent divergence²⁷. The unique primary structural features of Qa-2 suggest that it evolved before H-2K, -D and -L. Furthermore, TL may have diverged before Qa-2 because of the observed modest primary structural homology of TL with Qa-2, H-2K or H-2D (refs 12, 26 and M.J.S. et al., in preparation). Indeed, the unique structural features of the Qa-2 molecule and the finding of Qa and TL homologies in other species perhaps indicate that the Qa-2 and TL duplications occurred before speciation²⁸⁻³⁰. Alternatively, the multiple class I loci may have duplicated at approximately the same time and the observed structural differences evolved as a result of different selective pressures on the individual loci.

We thank Drs Mark Seigelman and J. Donald Capra for advice, discussion and technical support during these studies; Ms Patricia Wheeler and Dr Brad Ozanne for assistance in the two-dimensional gel analysis; Ms Sandy Graham, Shirley Shanahan, Kay Snave and Martha Miles for technical assistance; and Ms Daisi Marcoulides for typing the manuscript. This work was supported by NIH grants AI-13448 and AI-11851.

Received 14 January; accepted 3 March 1982.

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Mouse immunoglobulin allotypes: post-duplication divergence of γ 2a and γ 2b chain genes

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Sequence analysis of the mouse immunoglobulin heavy chain constant-region gene family has provided interesting clues to its evolutionary history1. There are eight genes, each consisting of four exons separated by noncoding sequences, which encode the three globular domains and the small hinge region of the constant-region polypeptide. The eight genes are believed to have arisen by duplications from a single ancestral gene and in particular, the γ 2a and γ 2b genes are thought to have been the result of a recent duplication²⁻⁵. They show, however, a rather surprising pattern of sequence divergence2-7. Schreier et al.7 have reported considerable sequence divergence between the two y2a alleles carried by the BALB/c and C57BL/6 inbred mouse strains; at many of the sites at which the two alleles differ, the BALB/c allele is identical to the BALB/c y2b gene. Thus it has been suggested that the BALB/c γ 2a gene has been converted by the $\gamma 2b$ gene. We have now sequenced the C57BL/6 y2b gene and report that the divergence between the two y2b alleles is very much less than that between the y 2a alleles and is consistent with point mutations. However, it is also clear that the third domain (CH3) of the C57BL/6 y2a gene has diverged so much from the homologous domain in the BALB/c γ 2a gene and the two γ 2b alleles that it may have derived from elsewhere in the heavy chain family and become inserted in the γ 2a gene by a double unequal crossing-over event.

The complete nucleotide sequence of the $\gamma 2b^b$ chain C gene, determined as described in Fig. 1 legend, is shown in Fig. 2. A total of 1,922 nucleotides were determined. The nucleotide sequence predicts the complete amino acid (336 residues) sequence encoded by the $\gamma 2b^b$ allele. We have compared the nucleotide sequence of the $\gamma 2b^a$ allele reported elsewhere⁸ with that of the y2bb allele for over 1,834 positions. As expected for alleles at a single genetic locus, the two sequences exhibit a high level of homology; the pattern of homology is given in Table 1. In the protein-coding regions, the two sequences differ by seven base transitions. There are four G→A transitions which lead to four amino acid substitutions: two located in the CH2 domain $[A \rightarrow G]$ at position 1,083 $(Gln \rightarrow Arg)$ and $A \rightarrow G$ at position 1,175 (Thr→Ala)] and two located in the CH3 domain [A \rightarrow G at position 1,604 (Asn \rightarrow Asp) and G \rightarrow A at position 1,609 (Met → Ile)]. There are three silent substitutions resulting from T o C transitions, one in each domain. Curiously, one of these is located adjacent to the A → G transition, leading to the amino acid change Thr -> Ala in the CH2 domain. It is highly probable that the double transition CA - TG leading to the Thr -> Ala change in the CH2 domain is the result of a double mutation in the y2ba gene as the same TG is found in the two γ 2a alleles.

It is generally assumed that intervening sequences (IVSs) and flanking sequences of a gene evolve more rapidly than the

Table 1 Comparison of nucleotide sequences of BALB/c and C57BL/6 γ2b and γ2a constant-region alleles

		$\gamma 2b^b/\gamma 2b^a$		$\gamma 2a^{\rm b}/\gamma 2a^{\rm a}$					
Gene segment	No. of transitions	No. of transversions	Deletions/insertions	Gene segment	No. of transitions	No. of transversions	Deletions/insertions		
CH1	1	· ·	-/-	CH1	0	2	poletions/ inscritions		
H			_/_	Н	3	2	-/- /15		
CH2	3	wow	-/-	CH2	6	5	-/15		
CH3	3	-	-/-	СНЗ	18	28	3/3		
3'UT		-	-/-	3'UT	8	7	-/-		

protein-coding sequence^{9,10}. It is interesting to note that the noncoding regions of both alleles are highly conserved with respect to size and sequence. There are only three base changes located in IVS1 and IVS2 and one in the 3' genomic flanking region, while the 3'-untranslated region is unchanged. Furthermore, the base deletion located in IVS1 is followed 16 nucleotides downstream by a base insertion, leaving the length of the IVS1 identical in both alleles.

The genetic polymorphism of mouse immunoglobulin in heavy chains has been extensively studied using anti-allotype allosera^{11,12}. However, in only a few cases have the structural basis and the precise location of these polymorphic determinants been determined¹³. By extensive specificity mapping, Herzenberg et al. 11 characterized six alleles at the γ 2b locus, the BALB/c γ 2b^a allele and the C57BL/6 γ 2b^b allele differing by three antigenic specificities. Because of limited amino acid variations between the products of both alleles, it is interesting to correlate the four amino acid differences to allotypic determinants. The first two amino acid changes (Glu \rightarrow Arg, Thr \rightarrow Ala), located in the CH2 domain, are separated by 31 amino acids, and thus are likely to determine separate specificities. A Thr \rightarrow Ala change also occurs in the same region of rabbit γ -chain, allowing the correlation of the A14 and A15 specificities with this amino acid change¹⁴. As the last two amino

acid changes are contiguous, it is reasonable to correlate the third allotypic specificity to the double change Asn-Met→ Asp-Ile (positions 1,604 and 1,609).

From our data and those of Schreier et al.⁷, it appears that the two closely linked $\gamma 2b$ and $\gamma 2a$ chain genes have different rates of evolution (Table 1). Whereas the two $\gamma 2b$ chain alleles differ by only 12 single base changes in 1,850 positions, the two $\gamma 2a$ alleles differ in 111 of 1,093 positions compared. The observation that some of the nucleotides that differ between the two $\gamma 2a$ alleles are conserved between $\gamma 2b$ and $\gamma 2a$ chain genes in BALB/c mice led Schreier et al.⁷ to postulate an intergenic conversion between the two genes. Our data show that these nucleotides are conserved between $\gamma 2a^a$, $\gamma 2b^a$ and $\gamma 2b^b$ genes. Furthermore, these positions are scattered along the sequence and not clustered as in the human fetal γ -globin genes¹⁵. Therefore, if there has been any gene conversion, it could have occurred only in the BALB/c ancestor.

The mutation rate of the $\gamma 2b$ chain gene is as expected for single base mutations in two alleles. However, the mutation rate in the $\gamma 2a$ gene, which is 20 times higher than that in the $\gamma 2b$ gene, is too great to be explained in this way. Note also that most of the differences between $\gamma 2a$ alleles are localized in the CH3 domain. Previous comparative studies of $\gamma 2a$ and $\gamma 2b$ genes led to the hypothesis that segments of the γ genes

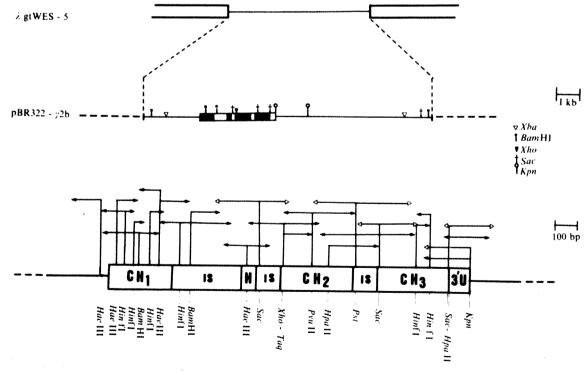


Fig. 1 Sequencing strategy for the mouse γ2b chain constant-region gene. As the γ2b gene lies on a 6.6 kilobase (kb) EcoRI fragment in C57BL/6 mouse embryo DNA¹⁶, EcoRI fragments (fractionated by sucrose centrifugation) ranging in size from 6 to 8 kb were ligated with AgtWES arms purified by gel electrophoresis¹⁷ and packaged in vitro. A hybridization probe consisting of a Xba-Kpn fragment of the BALB/c γ2b gene cloned in phage Charon 4A (unpublished results) was used to isolate a recombinant phage λgtWES-γ2b C57. For sequence analysis, the EcoRI insert was transformed to pBR322. A restriction enzyme map of this insert in pBR322 was determined by single and double digestion of DNA wth various enzymes. The strategy used for sequence determination is shown at the bottom. The extent and direction of sequencing of each fragment are indicated by the horizontal arrows: open and solid arrows represent 3' and 5' end-labelling, respectively. Only the restriction sites used as starting points are shown.

Fig. 2 Nucleotide and predicted amino acid sequences of the C57BL/6 y2b gene. The complete sequence of the DNA fragment containing all the constant regions (CH1, hinge, CH2 and CH3), the three intervening sequences (IVS1, IVS2 and IVS3) and the 5' and 3' flanking sequences is shown. Sequence determination was by the chemical degradation method¹⁸, five base-specific reactions (G, G+A, T+C, A>C) were used. End-labelling was done at the 3' protruding ends using terminal deoxy-nucleotidyl transferase with $[\alpha^{-32}P]$ cordycepin trisulphate, and at the 5' ends by exchange reaction using T4 polynucleotide kinase and $[\gamma^{-32}P]$ -ATP. The numbers below the sequence indicate the positions of the nucleotides. The predicted amino acid sequence of the translated Cy2n protein is shown above the DNA sequence. * The 10 divergent nucleotides found in y2b BALB/c are shown below the homologous bases in the γ 2b C57 sequence. ** Insertion at nucleotide 692 and deletion at nucleotide 708. *** Termination codon.

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ThrieuGlyCysteuValtysGlyTyrPheProGluSerValThrValThrTrpAsnSerGlySerLeuSerSerSerValHisThrPheProAlaLeuLeuGhnSerGlyLeuTyrThs
GACTCTGGGATGCCTGGTCAAGGGCTACTTCCCTGGAGTCAGTGACTTGGAACTCTGGAACTCTGGATCCTGCAGCACTTCCCAGCTCTCCCAGCTCTCCTGCAGTCTGGACTCTAGAC GBCLT-BEGGAT BCCT BBT CARBOSD THAT TECHTORIC TOWN TO THOMAT OF THE STATE OF THE STA 528 601 076
GluProSerGlyProlleSerThrlleAsnProCysProProCysLysGluCysHisLys
TCTCTCTGGGTTTGGTGCTTCTCTCCAAAAACCAGTAACATCCAGCCTTCTCTCTGCAGAGGCCCAGTGGAGCCCATTCAACAACCACTGTCCTCCATGCAAGAGTGTCACAAA
751 826 1576 TyrleuLysLysThr11eSerArgSerProGlyLys*** *poly(A)
GCAAAGCCTGGTACCATGTAAAACTGTCCTGGTTCTTTCCAAGGTATAGAGCATAGGTCTGGCCAGGGTTGGAGGACAGCCTTGTCTATAGGAAGAGAATGAGGTTT
1801 G 1876

have been exchanged between different subclass genes^{4,5}. As there is extensive divergence between the γ 2b and γ 2a CH3 domains and between the two allelic γ 2a CH3 domains, we suggest that the γ exons encoding γ 2a CH3 domains could have diverged on ancestral y-like genes before the y2b/y2aduplication. These exons, originally duplicated on a single chromosome, could behave as alleles as a result of different recombinational events (IVS-mediated transfer hypothesis or unequal crossing-over^{4,5}) in different mouse strains.

We therefore conclude from sequence analysis of the mouse γ -chain genes that the divergence rate between two homologous genes can be either accelerated or reduced by gene interchange: domain transfer increases genetic diversification while gene conversion maintains genetic stability.

We thank R. Nageotte for the C57BL/6 embryo DNA preparation; W. Roskam for the gift of \(\lambda\) gtWES arms; M. J. Singer, Mrs A. Minty and H. Eisen for critical reading of the manuscript; and B. Caudron for the computer program. This work was supported by grants from the CNRS (A.T.P. 3663 and 4247) and the Fondation pour la Recherche Médicale Francaise.

Received 8 September 1981; accepted 17 February 1982,

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A sex-linked enzyme in birds— **Z-chromosome conservation** but no dosage compensation

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In birds, the female is the heterogametic sex and the sexdetermining system is referred to as ZZ/ZW. In mammals the male is heterogametic, and the sex-determining system is referred to as XX/XY. The mammalian X chromosome appears to have been conserved largely intact during evolution. Thus the structural gene loci for glucose-6-phosphate dehydrogenase, phosphoglycerate kinase, α-galactosidase and hypoxanthine-guanine phosphoribosyltransferase are situated on the X chromosome of a wide variety of mammals. In addition, mammals show 'dosage compensation'; that is, although females possess two doses of each X-linked gene while males possess only one, females produce the same level of gene product as males. In birds, cytological studies and data on sex-linked morphological mutants suggest that the Z chromosomes of all birds are homologous, and that birds do not show dosage compensation for sex-linked genes. Ultimate proof of these hypotheses requires the discovery of proteins whose structural gene loci are encoded by the Z chromosome of birds1, none of which has previously been found. Here we report that the cytoplasmic isozyme of aconitase is Z-linked in the guinea fowl and probably Z-linked in the domestic fowl, house sparrow and two species of cockatoos, thereby providing evidence for Z-chromosome homology in birds. We also show that there is an apparent lack of dosage compensation for cytoplasmic aconitase in the domestic fowl, house sparrow and spotted turtledove.

Electrophoresis was conducted on Cellogel², and aconitase made visible by the method of Harris and Hopkinson³. Using this system, two zones of aconitase activity are found in bird liver. By differential centrifugation of mitochondrial and cytoplasmic fractions it was shown that the more anodally migrating zone of activity was the cytoplasmic fraction (Fig. 1). The cytoplasmic isozyme of aconitase (Acon_c) from liver of the guinea fowl (*Numida meleagris*—order Galliformes) migrates more anodally than that of domestic fowl (*Gallus domesticus*—order Galliformes) (Fig. 1). We denote the domestic fowl form of Acon_c as A and that of the guinea fowl as B. Hybrids between guinea fowl and domestic fowl were produced by artificial insemination⁴.

If the structural gene for Acon_c were autosomal, all offspring from such a cross would be heterozygous AB. If, however, the locus for Acon_c were on the Z chromosome, male offspring would be heterozygous AB whereas female offspring would be hemizygous A or B depending on the direction of the cross.

Crosses between female guinea fowl and male domestic fowl yielded hybrid embryos most of which died within the first week of incubation. A small number survived for longer periods, including one that survived to hatching. A total of 17 embryos were successfully typed for aconitase and of these, five were of known sex either from gonad histology or karyotyping⁵. Seven of the embryos were AB but ten were A (Table 1). Moreover,

Table 1 Cytoplasmic aconitase types of hybrid embryos between female guinea fowl (type B) and male domestic fowl (type A)

***************************************			******
		Acon _c type	
	Α	AB	В
Male embryos	0	3	0
Female embryos	2	0	0
Unsexed embryos	8	4	0
Total	10	7	0

of the five embryos of known sex, the three males were AB and the two females were A (Table 1 and Fig. 1). The results therefore indicate that the locus for Acon_c is not autosomal in the guinea fowl, but is located on the Z chromosome.

The data do not exclude the possibility that Acon_c is autosomally inherited in domestic fowl and sex-linked in guinea fowl. Crosses between female domestic fowls and male guinea fowls yielded embryos that died within 24 h. Aconitase activity was undetectable in most of these embryos. However, three embryos yielded a faint band of aconitase activity in the position of guinea fowl Acon_c and no detectable activity in the position of domestic fowl Acon_c. These results are expected for female hybrid embryos if Acon_c is encoded by a locus on the Z chromosome of the domestic fowl, and suggest therefore that Acon_c is Z-linked in the domestic fowl also.

We have found that Acon_e is polymorphic in three species of birds—the little corella, *Cacatua sanguinea* (order Psittaciformes), the yellow-tailed black cockatoo, *Calyptorhyncus funereus* (order Psittaciformes), and the house sparrow *Passer domesticus* (order Passeriformes).

Table 2 shows the observed phenotype frequences for Aconin little corellas from Three Springs, Western Australia. Of five males, two were type F (fast migrating band) and three were type FS (double banded fast and slow), whereas of 12 females, nine were type F and three were type S (Table 2). No heterozygous females were found. The appropriate statistical test is to construct a 2×2 contingency table for heterozygote and homozygote males versus females. Applying Fisher's exact probability, P is 1.5%. The absence of heterozygous females is therefore statistically significant. If we take the gene frequencies for males and calculate expected phenotype frequencies for females on the hypothesis that $Acon_c$ is sex-linked, the fit to the observed results is then excellent (Table 2), suggesting that the locus for $Acon_c$ is on the Z chromosome of C. sanguinea.

In five yellow-tailed black cockatoos (two males and three females) from Bool Lagoon, South Australia, three alleles for Acon_c were found, designated F, I and S (fast, intermediate and slow). One male was heterozygous FI, and the other was heterozygous IS, but of the three females, one was single-banded F, one was single-banded I and the third was single-banded S. Again these data are more consistent with the locus for Acon_c being on the Z chromosomes of *C. funereus* than on an autosome.

Samples of house sparrows (*P. domesticus*) from Adelaide, Canada and the UK were also polymorphic for Acon_c (data for Canada and UK from S. R. Cole and D. Parkin, personal communication). Again there was an absence of heterozygous females which is highly significant statistically in the UK sample, and significant at the 5% level in the Canadian sample (Table 2). The observed phenotype distributions for females are, on the other hand, consistent with the locus for Acon_c being on the Z chromosome of *P. domesticus*.

Ohno¹ has suggested that the Z chromosome has been conserved during evolution of the class Aves; thus any gene that is on the Z chromosome of one species of bird should be found on the Z chromosome of all bird species. The present study has shown that Acon_c is on the Z chromosome of a galliform and probably on the Z chromosome of another galliform, two psittaciforms and a passeriform. These data provide support for Ohno's hypothesis and suggest that Acon_c is on the Z chromosome of all birds. The data do not prove, however, that the entire Z chromosome has been conserved, rather that part of the Z chromosome carrying the structural locus for Acon_c has been conserved.

Ohno¹ also suggested that the Z chromosome of birds may be homologous with the X chromosome of mammals. Clearly, this is not the case. The enzymes glucose-6-phosphate dehydrogenase and phosphoglycerate kinase are sex-linked in

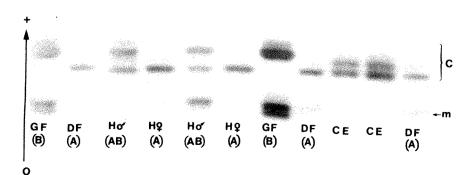


Fig. 1 Electrophoretic gel stained for aconitase. O, origin; m, mitochondrial fraction (Acon_m); C, cytoplasmic fraction (Acon_c); GF, guinea fowl (type B for Acon_c); DF, domestic fowl (type A for Acon_c); H, hybrid between female guinea fowl and male domestic fowl; CE, cytoplasmic extract of chicken liver after differential centrifugation to remove the mitochondrial fraction. Note that the proposed heterozygotes are double-banded, indicating that avian cytoplasmic aconitase is monomeric as in mammals³. The weaker secondary bands result from post-translational modification.

Table 2 Phenotype frequencies for Acon_e by sex in little corellas from Three Springs, Western Australia, and in house sparrows from UK, Canada and Adelaide

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	a, Little corella			F	FS	s	Total	c, House sp	parrows—Ca	inada	F	FS	S	Total
P = 0.7 Expected Autosomal S.9 S.0 1.1 P = 0.167 Expected Expected Sex-linked Sex-lin	Observ								Observed:		2 5			39 21
Hetero-zygote Zygote Total Hetero-zygote Zygote Total Hetero-zygote Zygote Total Hetero-zygote Zygote Zygote Total Hetero-zygote Zygote Total Hetero-zygote Zygote		ted	Autosomal	5.9	5.0	1.1	12			Autosomal	0.6			
Male 3 12 12 12 Female 0 21 21	, 0.0 ,0			Hetero-			Total							Total
P = 1.5% (exact test) $P = 1.5% (exact test)$														39 21
P = 1.5% (exact test) $P = 1.5% (exact test)$				3	1.	4	17				9	51		60
F FS S Total F FS S Total F FS S Total F FS S Total				<i>P</i> =	1.5% (e	xact tes	t)				P=1	.5% (e	xact te	st)
Disserved: Male	b, House sparre	ows—UI	Κ	F	FS	s	Total	d, House	sparrows—A	delaide	F	FS :	s	Tota
December 20,086 Expected Autosomal 1.21 25.62 136.2 q = 0.914 female: Sex-linked 14.00 0 149.0 p = 0.107 Expected Autosomal 0.2 3.6 15.2 q = 0.893 female: Sex-linked 2.0 0 17.0	Ob	bserved:							Observed:					14 19
Male 39 188 227 Male 3 11 Female 0 163 163 163 39 351 390 3 30 30 3 670 (constant)			Autosomal	1.21										
Male Female 39 168 227 168 227 168 163 163 Female 0 19 39 351 390 3 30 168 169 169 169 169 169 169 169 169 169 169							Total							Total
39 351 390														14 19
P = 6.7% (exact test)				39	3:	51	390				3		30	33
$P \ll 0.01\%$ (exact test)				P ≪ ().01% (e	exact tes	st)				P = 0	5.7% (e	exact t	est)

mammals but not birds⁶. Acon_c, on the other hand, is sex-linked in birds, but not mammals³.

Because Acon_c is Z-linked in birds, males have two doses of the gene and females one dose. Therefore in the absence of dosage compensation, male birds will possess twice the activity of cytoplasmic aconitase of females. We have measured the level of cytoplasmic aconitase activity in the livers of 1-day-old chickens, mature house sparrows and mature spotted turtle-doves (Streptopelia chinensis—order Columbiformes). In all three species, females had statistically significantly lower levels of cytoplasmic aconitase activity than males (Table 3). On the

other hand, where the null hypothesis was tested that males possess twice the activity of females, non of the P values were statistically significant (Table 3). That such a result was not due to generally lower enzyme activity in females was shown by comparing the levels of isocitrate dehydrogenase (IDH) activity in the same cytoplasmic fractions. In all three species, males and females did not differ significantly with respect to IDH activity. It is clear, therefore, that the locus for Acon_c does not show total dosage compensation in birds.

Dosage compensation of all but one or two X-linked genes in mammals is effected by inactivation in each cell of one of

Table 3 Aconitase activity in the supernatant of liver homogenates of males and females of three species of birds representing three orders

				Nu	Il hypothes $\mu_1 = \mu_2$			hypothes $\mu_1 = 2\mu_2$	
Species	Sex	N	Aconitase activity	1*	d.f.	P	ı	d.f.	P
P. domesticus (Passeriformes)	F M	5 5	0.095 ± 0.023 0.135 ± 0.016	3.11	7.2	<1%	2.58	4.4	>5%
G. domesticus (Galliformes)	F M	4 5	$0.032 \pm 0.005 \\ 0.051 \pm 0.012$	3.01	5.7	<2.5%	1.84	6.9	>20%
S. chinensis (Columbiformes)	F M	4 8	$0.034 \pm 0.004 \\ 0.081 \pm 0.025$	3.67	7.9	<0.5%	1.24	9,6	>20%

Livers were removed from freshly killed individuals and placed in twice their weight of cold homogenizing solution (0.25 M sucrose, 0.001 M EDTA in 0.1 M Tris-HCl pH 7.8). The tissues were homogenized by hand and centrifuged at 10,000g at 4 °C for 25 min. The supernatant was removed and stored frozen at -80 °C. Electrophoresis of the supernatant revealed only a trace of the mitochondrial isozyme of aconitase (Fig. 1). Aconitase activity in the supernatant was assayed by a modification of the method of Ruse and O'Connell¹⁰, isocitrate dehydrogenase activity by the method of Cleland¹¹, and total protein by the method of Lowry¹². Values are mean ± s.d. µmol NADP converted per min per mg protein. N, number of individuals.

the X chromosomes?. In Drosophila most genes on the X chromosome are dosage-compensated, but here each gene on the X chromosome produces half the amount of product in females as in males8. However, not all genes on the X chromosome of Drosophila show complete dosage compensation. Therefore the observation that Acon, shows no dosage compensation in birds should not be taken as proof that all Z-linked genes in birds show no dosage compensation. Nevertheless, when taken in conjunction with the morphological data1, the present data suggest that lack of dosage compensation is the rule in birds. As pointed out by Johnson and Turner⁹, it may be no coincidence that the groups known to show dosage compensation (mammals and Drosophila) have XX/XY sex determination while the groups lacking dosage compensation (lepidopterans and birds) have ZZ/ZW sex determination.

For their help with various aspects of this work we thank Drs D. Briscoe, R. Holmes, C. H. S. Watts, B. J. Richardson, D. Hayman, D. Saunders, G. Smith, D. Parkin, R. Hope, Ms C. Lynch, Messrs T. Benson, J. Bourne and S. R. Cole.

Received 7 October 1981; accepted 5 March 1982.

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Evidence for X-linkage and non-inactivation of steroid sulphatase locus in wood lemming

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One of the two X chromosomes is inactivated in somatic cells of adult female mammals to compensate for unequal amounts of X-chromosomal genes in the two sexes1. Although the general validity of this concept is not in doubt, there is evidence that a segment of the distal short arm of the human X chromosome carrying the gene, or genes for the Xg blood group system, the steroid sulphatase (STS) locus^{2,3}, and a gene controlling a serologically defined male-specific antigen^{4,5}, is never inactivated. It is not known whether this non-inactivated segment is a special feature of the human X chromosome or whether it is a general feature of the ancestral, highly conserved, mammalian X chromosome⁶. In man the number of functional STS gene copies can be deduced from intracellular STS activity7. With this in mind we have investigated the number of active gene copies on the X chromosome of the wood lemming (Myopus schisticolor, Lilljeborg), by assaying STS in cultured cells of this species. We report here that STS activity is directly correlated to the number of X chromosomes and unrelated to the phenotypic sex. This suggests that in the wood lemming the STS gene is also X-linked and not subject to inactivation.

The remarkable stability of the mammalian X chromosome can be explained, in part, by the fact that males carrying X-autosomal translocations are mostly sterile. This is thought to result from a failure of inactivation of translocated X-chromosome segments during gametogenesis⁸. As a result, Xautosomal translocations cannot be transmitted through males, and therefore do not spread in the population. This notion suggests that strict evolutionary conservation may be confined to those parts of the X chromosome that are subject to inactivation, as males with translocations involving permanently active segments would not necessarily be sterile. In man the existence of a non-inactivated segment on the distal short arm of the X chromosome is now well established9. For one of the three genes assigned to this region (that is, the STS gene), in both sexes, significant differences in enzyme activity have been demonstrated for various cells and tissues such as fibroblasts, hair roots and placenta^{7,10,11}. Similar gene-dosage relationships have been observed in numerical, and most structural, Xchromosomal aberrations¹²

In the wood lemming, numerical sex chromosome aberrations are relatively frequent due to its unique system of sex determination. There are two different types of X chromosome, X and X*, which can be distinguished cytogenetically¹³. Specimens with XX, X*X, X*Y, XO and X*O sex chromosome constitutions are phenotypic females and are fertile. Males have the XY sex chromosomes^{13,14}. In germ-line cells of X*Y females only the X* chromosome is found. By a mechanism of mitotic double non-disjunction, the Y chromosome is replaced by a copy of the X* (refs 15, 16). The relatively high incidence of numerical sex chromosome aberrations probably depends on occasional failure of this non-disjunction mechanism.

The molecular basis of sex reversal in X*Y females is not yet understood. The fact that the X* differs from the X both morphologically and functionally suggests that this may be due to duplication or deletion of genes involved in sex differentiation. In man, a gene or genes controlling sex differentiation may be closely linked to the STS locus on the non-inactivated distal part of Xp^{4,5,17,18}. To determine whether in a rodent species the STS gene is also X-linked and not inactivated, and whether the dosage of this gene is altered on the rearranged X*, we have determined STS activity in cultured fibroblasts from wood lemmings of seven different sex chromosome constitutions.

Primary fibroblast cultures were established from heart tissues in Earle's modified Eagle's minimal essential medium supplemented with 15% fetal calf serum. After karyotyping and two or three further passages in culture, the cells were analysed biochemically. In three subsequent series of experiments, a total of 54 cultures, representing 27 wood lemming specimens, were tested for STS activity; 30 cultures were also assayed for an X-linked reference enzyme, that is, glucose-6phosphate dehydrogenase (G6PD).

STS activities were found to be directly correlated with the number of X chromosomes, irrespective of the phenotypic sex (Table 1) while no such correlation was found for G6PD (Table 2). The simplest interpretation of these results is that in the wood lemming, as well as in man, STS is X-linked and not subject to X-inactivation. Therefore, we suggest that the STS gene may be part of the conserved X-chromosomal linkage group of mammals, although this has yet to be confirmed in other mammalian species. Conservation of a permanently active segment of the mammalian X chromosome could be related to the hypothesized homology between this segment and part of the Y chromosome^{19,20} for which there is now increasing, although circumstantial, evidence^{17,18,21}. It is noteworthy that in man, genes on this segment have been implicated in sex determination^{4.5,17,18}, suggesting that sex reversal in the wood lemming may be due to altered dosage of related genes on the rearranged X*. While this possibility cannot be excluded, the observation that STS levels are almost identical in wood lemming specimens, whether they carry the normal X or the altered

Table 1 Steroid sulphatase activity in cultured fibroblasts of wood lemmings with different sex-chromosome constitutions

Animal no.	Phenotypic sex	Sex chromosomes	No.	pt 1 of X osomes	No.	pt 2 of X osomes	No	of X
			1	2	1	2	1	2
593	<i>ਹੈ</i>	XY			206*			
598	ੈ	XY	240		355			
4,849	♂	XY			*		279	
4,988	ð	XY					446	
4,360	♂	XY					442	
4,992	₫	XY					390	
4,656	♂	XY					434	N
597	φ	XX		563		827		
600	φ	XX		589				
5,148	Q.	XX						1261
5,212	Q	XX						679
5,089	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	XX						917
4,985	Ŷ	XX						1026
5,211	ð	XX						773
594	Q	X*Y			145*			
599	Ş	X*Y	223					
5,038	Ş	X*Y					547	
4,994	Q	X*Y					340	
5,020	Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q	X*Y					455	
5,091	Q	X*Y					295	
5,019	Ş	X*Y					444	
592	Ş	X*X		733				
596	Q.	X*X		597		813		
595	Q	XO	245		397			
591	Q	X*O	235					
587	Q	X*XY				834		
589	ðt	X*XY				528		
lean ± s.d.			235±5	621 ± 38	376‡	751 ± 74	407 ± 26	931 ± 102
atio 1X:2X			1/2	2.65	1/2	2.00	1/	2.28
lean protein co ug per 50 µl of			146	171	158‡	180	211	291

STS activity is expressed as pmol dehydroepiandrosterone sulphate cleaved per mg protein per h. For determination of STS, early confluent cells were collected with trypsin, washed twice in Hank's balanced salt solution, suspended in lysis buffer (5 mM Tris-HCl, 2 mM EDTA, 7 mM mercaptoethanol, 0.1% Triton X-100 pH 7.5) and sonicated using a Branson sonifier with microtip (4×5 s, 50 W). ³H-dehydroepiandrosterone sulphate (DHEAS, specific activity 24 mCi μ mol⁻¹; NEN) was diluted with cold DHEAS to a specific activity of 10μ Ci μ mol⁻¹, then extracted three times with diethyl ether to remove free dehydroepiandrosterone. 10μ l of this solution, corresponding to 10μ 0 nmol DHEAS, were incubated at 37 °C for 2μ 1 with 50μ 1 of homogenate and 50μ 1 of 0.1μ 1 M Tris-HCl buffer pH 7.5, containing 10μ 2 mg bovine serum albumin (BSA) per ml. Then 0.1 ml of 0.2 M NaOH and a 10-fold excess of cold DHEAS were added, followed by extraction with 3 vol of ether. An aliquot of the ether phase was evaporated to dryness, and radioactivity determined by scintillation counting²². Blank controls contained lysis buffer with 10 mg ml⁻¹ BSA instead of homogenate. The relationship between STS activity and protein concentration is linear $(r = 0.99 \pm 0.07)$ up to 800 µg of protein. Mean enzyme levels can vary in subsequent tests due to non-saturating substrate concentrations in the assay. Each value represents the mean of two independent cultures which were each tested in duplicate

Cultures not confluent. † Sterile male. ± Confluent cultures only.

X* chromosome, strongly argues against a duplication or deletion of the STS locus on the abnormal X*. It follows that, in this species, either the STS gene is not closely linked to gene(s) responsible for sex reversal, or the two genes have been separated by rearrangement on the aberrant X* chromosome.

We thank Professor Karl Fredga for providing material for this study; A. Gullberg, K. Jönsson, B. Müller-Migl, U. Strohmaier, M. Sunner and J. Zimmer for technical help; and Professor Ulrich Wolf for reading the manuscript. This work was

Table 2 G6PD activity (mU per mg protein) in wood lemming cultures with XY, X*Y and XX sex chromosome constitutions

XY		X*Y		XX
	Animal		Animal	
	no.		no.	
44.5	4,994	33	4,985	47
38.5	5,031	47	5,148	54
47	5,020	44	5,211	31
44	5,091	49	5,089	44
49	5,019	47	5,212	57
44.6 ± 1.8		44.0 ± 2.9		46.6 ± 4.5
	44.3 ± 1.6			46.6 ± 4.5
	44.5 38.5 47 44 49	Animal no. 44.5 4,994 38.5 5,031 47 5,020 44 5,091 49 5,019 44.6±1.8	Animal no. 44.5 4,994 33 38.5 5,031 47 47 5,020 44 44 5,091 49 49 5,019 47 44.6±1.8 44.0±2.9	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

G6PD was assayed according to Löhr and Waller²³. Each value represents the mean of two independent cultures which were each tested twice.

supported by grants from the Deutsche Forschungsgemeinschaft, the Swedish Natural Science Research Council and the Deutsche Akademischer Austauschdienst.

Received 25 November 1981; accepted 5 March 1982.

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Transposition of a tandem duplication of yeast mating-type genes

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The switching of mating-type genes in both homothallic and heterothallic strains of the yeast Saccharomyces cerevisiae involves a site-specific transposition event1-3. A sequence at the mating-type locus (MAT) is replaced by a copy of a or α information from the unexpressed loci, HML or HMR. Although MATa and MATa contain unique sequences of ~700 base pairs (bp), they are flanked by sequences that are also found at HML and HMR4.5 (Fig. 1a). Recent results6-8 have suggested that the switching of \overline{MAT} alleles involves an intrachromosomal mitotic gene conversion event, in which a donor sequence at HML or HMR pairs with the homologous sequences at the MAT locus. On this view the switching of mating-type information must be able to accommodate the region of non-homology in a heteroduplex structure. (Analogous gene conversions of non-homologous regions have been well documented during meiosis at MAT and for deletions in other yeast genes^{9,10}.) One prediction of a gene conversion mechanism is that it should be possible to transpose a tandem duplication of mating-type genes from HMR to MAT. In such a switch, pairing would occur between MAT and homologous sequences flanking the two copies of the tandem duplication at HMR. Our results reported here demonstrate that a tandem duplication created at HMR by recombinant DNA techniques can be transposed to MAT without the loss of these sequences at the donor locus.

Tandem duplications of mating-type genes at HMR were constructed by transforming the heterothallic (ho) strain DB 745 (HML\alpha MAT\alpha HMR\alpha ura3-52 leu2 ade1) with plasmid pDRMATa3, which contains the 3.5 kilobase (kb) EcoRI-HindIII MATa fragment and the 1.1 kb HindIII URA3 fragment inserted into pBR322 (Fig. 1a). In 3 of 20 Ura+ transformants the plasmid had integrated at HMR. These were shown by Southern blot analysis to be two types. In DR103 and DR107 a single copy of the plasmid was inserted, generating the structure HMR::(MATa-URA3-pBR322) (Fig. 1b). At least one of the two fusions between MAT and HMR sequences expressed a information, so that the $MAT\alpha$ cell was phenotypically non-mating. The third transformant had two copies of the plasmid tandemly inserted. generating the structure HMR: $(MATa-URA3-pBR322)_2$ (Fig. 1c). Again, at least one of the a-containing regions expressed a information; thus strain DR204 was also non-mating. We have also obtained an insertion of pDRMATa3 at MATa, giving the structure MATa-URA3-pBR322-MATa (strain DR212, Fig. 1d). This duplication has the structure expected for the transposition of a tandem duplication for HMR::(MATa-URA3-pBR322).

Figure 2a shows an example of the Southern blot analysis used to confirm these structures. A BamHI digest of DNA from the transformants was probed with labelled pDRMATa3 (Fig. 2a, lanes 1-4) or with labelled pBR322 (lanes 5-8). Because there is only a single BamHI site in pDRMATa3 (Fig. 1), one would expect a single integrant of the plasmid to yield two BamHI fragments, one of which should hybridize strongly with pBR322, and the other, homologous to only 300 bp (Fig. 1b), weakly. This was indeed the case for DNA from transformants DR103 and DR107 (Fig. 2a, lanes 6, 7). The third transformant, DR204, gave rise to the same BamHI fragments plus one strongly hybridizing fragment the same size as the original plasmid (Fig. 2a, lane 8). This is consistent with the HMRa::(MATa-URA3-pBR322)₂ structure shown in Fig. 1c. The tandem duplication at MAT (strain DR212) results in differently sized BamHI fragments (Fig. 2a, lane 1).

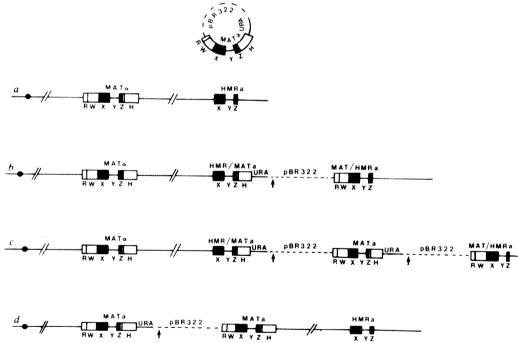
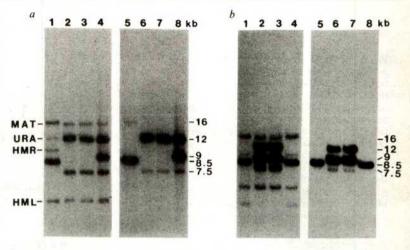


Fig. 1 Structures generated by the integration of plasmid pDRMATa3 by homologous recombination at HMRa or MATa. The plasmid contains the 3.5 kb EcoRI-HindIII fragment including MATa and the 1.1 kb HindIII fragment of URA3. a, The regions of homology between the plasmids and HMRa are indicated as solid blocks (X, Y and Z, according to ref. 5). The regions of homology between the plasmid and MATa are shown by the open and solid blocks [the W region plus the adjacent segments extending to the EcoRI site (R) and the HindIII site (H)]. The HMLa locus on the left arm of the chromosome (not shown) shares with MAT the homology regions W, X and Z. The Ya region at MATa is not homologous to Ya in the plasmid or at HMRa. The pBR322 sequences are indicated as ---. BamHI sites in pBR322 used to analyse the structure of the transformants are shown by arrows. b, A single integration of the plasmid at HMRa (strain DR103 and DR107). c, A tandem insertion of the plasmid at HMRa (strain DR204). d, Insertion of the plasmid at MATa (strain DR212).

Fig. 2 Southern blot analysis of a transposition of a tandem duplication of a-mating-type sequences from HMRa::(MATa-URA3)₂ to replace MATα. a, Analysis of insertions of pDRMATa3 at HMRa and MATa. DNAs from four strains, described below, were digested eith BamHI, separated by agarose gel electrophoresis and the Southern blot probed with either labelled pDR MATa3 (lanes 1-4) or labelled pBR322 (lanes 5-8). Lanes 1, 5: strain DR212 (Fig. 1d), MATa::(MATa-URA3); lanes 2, 6: strain DR103 (Fig. 1b), HMRa::(MATa-URA3); lanes 3, 7: strain DR107 (Fig. 1b), HMRa::(MATa-URA3); lanes 4, 8: strain DR204 (Fig. 1c), HMRa::(MATa-URA3)2. The identity of each band was determined by additional hybridizations with DNA probes specific for URA3, MAT and HML (data not shown). Two BamHI fragments of 12 kb and 7.5 kb are generated when pDRMATa3 integrates at HMRa (see Fig. 1b and lanes 2, 3, 6, 7). A tandem insertion of the plasmid at HMRa creates the same two BamHI fragments, plus a 9 kb fragment identical in size to the plasmid (see Fig. 1c and lanes 4, 8). Creation of a tandem duplication at MATa (by the integration of plasmid pDRMATa3 at MATa) creates two RamHI fragments of 16 and 8.5 kb, both of which hybridize to labelled pBR322. b, Southern blot analysis of four meiotic segregants from a diploid (DR204/A145 no. 2) of genotype:



HMLα MATa URA3 pBR322 MATa HMRa::(MATa-URA3)₂ HMLα MATα HMRa

DNA from each of four segregants was cut with BamHI and the separated fragments probed with labelled pBR322 (lanes 1-4) or pDRMATa3 (lanes 5-8). Two of the segregants were a-mating and Ura⁺, and carry MATa sequences derived by transposition from $HMRa::(MATa-URA3)_2$. These two strains (lanes 1, 4; lanes 5, 8) contain BamHI fragments characteristic of a tandem duplication at MATa (see Fig. 2a, lanes 4, 8). The other two segregants (lanes 2, 3; lanes 6, 7) were non-mating, Ura⁺ and carry MATa as well as a information expressed at $HMRa::(MATa-URA3)_2$. The BamHI fragments are identical in size to those in the original parent strain, DR204 (Fig. 2a, lanes 3, 7).

Transposition of copies of a information HMRa::(MATa-URA3-pBR322) was monitored by crossing the non-mating Ura+ strains with strain A145 (ho MATa ura3 leu1 ade1) and selecting Ura+ diploids. These could only arise if the non-mating strains became a-mating by the transposition of a information from the tandem duplication at HMR to replace $MAT\alpha$. Ten diploids from each of the three mating experiments were analysed by tetrad analysis to determine whether the conversion of the MATα HMRa::(MATa-URA3-pBR322) strain to MATa resulted from an event that also transposed a copy of the URA3 gene (see Table 1). In all 20 cases involving strains DR103 and DR107 the segregation of markers indicated that a single copy of MATa had been transposed to the MAT locus without URA3—that is, the expression of an a mating type was not linked to URA3 and each tetrad contained two Ura+ and two Ura- segregants. If there were two unlinked URA3 genes segregating in these cases, the probability of finding a 2+:2- tetrad would be only 1/6. Furthermore, there were an equal number of α -mating Ura strains and non-mating Ura segregants, presumably carrying $MAT\alpha$ and either HMRa or HMRa::(MATa-URA3-pBR322) respectively. In contrast, in two of the ten diploids isolated from mating strain DR204 with A145 there was a second copy of URA3 segregating in the cross and one copy of URA3 was tightly linked to MATa. This is evident from the fact that most tetrads contained three or four Ura segregants per tetrad and that every a-mating segregant was Ura⁺. Half of the $MAT\alpha$ segregants were Ura⁺ and non-mating. We concluded that in two of the ten transpositions of a information from HMRa:: (MATa-URA3-pBR322)2 in strain DR204 the transposition event also mobilized the URA3 gene.

Using Southern blot analysis to study one transposition event (DR204/A145 no. 2), we have shown that (1) the transposition did indeed create a tandem duplication of *MATa* flanking *URA3* and pBR322 at *MAT*; and (2) that the structure at the donor *HMRa*::(*MATa-URA3*-pBR322)₂ locus was not altered (see Fig. 2b). DNA was isolated from one tetrad of diploid DR204/A145 no. 2 in which all four segregants were Ura⁺. A Southern blot of DNA digested with the restriction endonuclease *BamHI* was probed with labelled pDR*MATa3* (Fig. 2b, lanes 1-4) or pBR322 (lanes 5-8). The two segregants that were a-mating and Ura⁺ contained two new bands (16 and 8.5 kb) characteristic of an insertion of the *MATa-URA3*-pBR322 plasmid at *MATa* (lanes 1, 4). The other two

segregants which were non-mating and Ura⁺ were shown to carry $MAT\alpha$ and the pair of 7.5 and 12 kb BamHI restriction fragments found with the $HMR::(MATa-URA3-pBR322)_2$ duplication of the parent strain DR204 (lanes 2, 3). These data confirm that the transposition event created a tandem duplication of MATa and transposed the intervening URA3 and pBR322 sequences.

These experiments show that the mechanism of switching MAT genes can transpose a tandem duplication of mating-type genes and 5.5 kb of non-homologous intervening sequences. If the evidence favouring a gene conversion model for MAT switching is correct, even the transposition of a single copy of a to replace $MAT\alpha$ must form a heteroduplex in which the non-homologous sequences of a- and α -specific DNA are not paired. In the case where a tandem duplication of MAT genes was transposed, we presume that a heteroduplex formed between homologous sequences (W and Z) on the outside

Table 1 Heterothallic conversions of $MAT\alpha$ to MATa or to MATa-URA3-pBR322-MATa

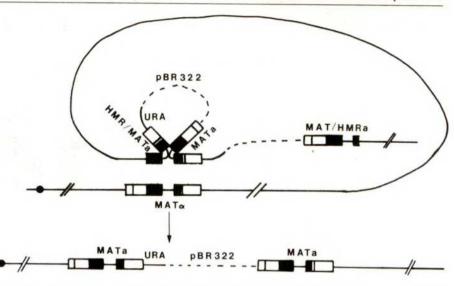
					Tetra	d type	2			
	Mat	Ura	Mat	Ura	Mat	Ura	Mat	Ura	Mat	Ura
	a	_	a	+	a	+	a	+	a	+
	a	-	a	-	a	+	a	+	a	+
	NM	+	α	_	α	-	α	_	NM	+
Diploid	NM	+	NM	+	α	+	NM	+	NM	+
DR103/A145*	8		3	6	1	3				
DR107/A145*	7	8	3	7	1	0				
DR204/A145†	5		2	6		8				
No. 2						1		8	1	
No. 8						3	1	1	2	

Heterothallic non-mating strains DR1203 and DR107 (ho, MAT α , HMRa::(MAT α -URA3-pBR322)) and strain DR204 (ho, MAT α , HMRa::(MATa-URA3-pBR322) $_2$) were crossed with the MAT α HMRa ura3 strain A145. Ten independent rare matings of each cross were purified, sporulated and analysed by tetrad dissection. The total numbers of each type of tetrad are shown. Non-mating segregants are designated NM.

^{*} Sum of 10 independent diploids analysed.

[†] Sum of eight independent diploids analysed.

Fig. 3 A model for the transposition of a tandem duplication of mating type genes from HMR::(MATa-URA3-pBR322)2 to replace MATa. A gene conversion event in which sequences at HMR are used to replace non-homologous sequences at MAT first requires the homologous pairing of these regions, as shown. Instead of the normal 0.7 kb region of non-homology transferred between HMRa and MATa, the pairing of the outside homologous regions of the tandem duplication at HMRa::(MATa-URA3pBR322)2 leads to the insertion of a 7.9 kb region of DNA not homologous to $Y\alpha$.



portions of two different donor sequences (Fig. 3) could then create a large non-homologous region in the middle of the heteroduplex. DNA mismatch repair favouring the incoming strand would then allow the entire region to be transposed. It is significant that we found such transpositions with strain DR204 but not with strain DR103 or DR107. One explanation for this difference is that the extent of homologous pairing that can be formed, especially on the right side of the unique sequences, is substantially greater (1.1 kb compared 0.23 kbusing the multiple tandem cation HMRa::(MATa-URA3-pBR322)2 than the single HMRa::(MATa-URA3-pBR322) integrant. The greater homology may stabilize the unusually large non-homologous region in a heteroduplex structure.

We have recently also carried out a complementary study of the switching of a tandem duplication of MAT genes at the mating-type locus (J.E.H. and D.T.R., in preparation). Homothallic strains carrying a $MAT\alpha$ duplication switched efficiently and usually the tandem duplication was replaced by a single MATa locus. Pedigree studies have shown that the loss of the duplication and the switch to the opposite mating type occurs during a single cell division. Analogous to our interpretation of transposition of a duplication from HMR to MAT (Fig. 3), we explain the complementary study by supposing that the donor HMRa must pair with the common region X, to the left of one $MAT\alpha$ gene and the common region Z1 on the right of the other $MAT\alpha$ locus. A gene conversion would then replace a large (7.9 kb) non-homologous sequence with the 0.7 kb unique a sequence.

We believe our results are consistent with an intrachromosomal gene conversion mechanism for switching mating type.

This research was supported by USPHS grant GM20056. We thank Barbara Weiffenbach for her comments on the manuscript. D.T.R. was a Charles A. King Trust Research Fellow.

Received 14 September 1981; accepted 23 February 1982.

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Induction of a DNA glycosylase for N-methylated purines is part of the adaptive response to alkylating agents

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The resistance of Escherichia coli to simple alkylating agents, for example N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), is markedly increased in cells previously exposed to low doses of the agents. This adaptive response seems to reflect improved repair of alkylation lesions in DNA, and cells become less sensitive to both the mutagenic and killing effects of alkylating agents^{1,2}. Adaptation to the former is due largely to the induction of a repair function that removes O6-methylguanine (m⁶G) from DNA^{3,4}. This activity has been purified from induced cells and is attributed to a protein that transfers the methyl group of an m6G residue in DNA to one of its own cysteine residues5. Consequently, no excision of damaged nucleotide residues seems to be necessary. The adaptive response to killing by alkylating agents, however, is less understood. DNA excision-repair with gap-filling catalysed by DNA polymerase I seems to occur as part of the inducible response, as it depends on the presence of a functional polA+ gene2 and enhanced DNA polymerase I-mediated repair synthesis has been observed in adapted cells challenged with MNNG6. Here we show that the adaptive response involves induction of not only a methyltransferase for m6G, but also a DNA glycosylase that catalyses the release of purines methylated at ring nitrogen atoms. We also identify the minor alkylation product 3-methylguanine (m3G) as an important lethal lesion.

One of the major methylation products in DNA, 3-methyladenine (m3A), is known to be actively and rapidly removed in vivo 7.8. The enzyme responsible, 3-methyladenine DNA glycosylase I, can easily be detected in extracts from unadapted cells, and it seems to be constitutively expressed. This activity has been extensively purified and characterized9. The enzyme, which has a molecular weight of about 19,000, shows a very narrow substrate specificity and of several different DNA methylation products, only m3A is released from methylated DNA. E. coli mutants (tagA) deficient in this enzyme have been isolated, and although they are less able to remove m3A from their DNA after exposure to alkylating agents8, they are not totally deficient in m3A DNA glycosylase activity. The

Table 1 DNA glycosylase activities in various E. coli strains before and after adaptation by MNNG treatment

		Specific acti extract (µ	
E. coli strain	DNA Glycosylase	Unadapted	Adapted
E. coli B/r	Uracil	3,800	3,500
	Hypoxanthine	2.1	2.0
	3-Methyladenine I	3.6	3.6
	3-Methyladenine II	0.22	4.1
	3-Methylguanine	~0.05	1.1
	7-Methylguanine	~0.02	0.2
	Formamidopyrimidine	4.2	3.9
	Thymine glycol	1.9	1.7
	Urea	5.5	4.8
E. coli BK2114	3-Methyladenine I	< 0.02	< 0.1
(tagA)	3-Methyladenine II	0.20	7.8
	3-Methylguanine	~0.05	1.7
	7-Methylguanine	~0.02	0.4

E. coli cells were adapted by growth in MNNG (1 μg ml⁻¹) for 60 min. The uracil, hypoxanthine, formamidopyrimidine, thymine glycol and urea DNA glycosylases were assayed in cell-free extracts as described previously^{13,22-25}. The products released by m³G, m⁷G and m³A DNA glycosylases from ³H-dimethylsulphate-treated DNA were characterized by HPLC as described in Fig. 2 legend.

residual function, which represents 5-10% of the total activity in wild-type cell extracts, has properties different from m³A DNA glycosylase I: it is resistant to product inhibition by free m³A; it is relatively heat resistant; and it is present in similar amounts in wild-type cells and in *tagA* mutants. It has therefore been concluded that the minor activity is a separate enzyme, which has been termed 3-methyladenine DNA glycosylase II^{8.10}.

When the enzymatic release of free m3A from alkylated DNA was measured, using cell extracts from unadapted and adapted E. coli, the adapted cells exhibited two- to threefold more DNA glycosylase activity. To assess the relative contributions of m³A DNA glycosylases I and II, the cell extracts were chromatographed on Sephadex G-75, and column fractions assayed in the presence and absence of m³A (3 mM). In these conditions, m³A DNA glycosylase I is 90-95% inhibited whereas enzyme II is not affected. Figure 1a shows that enzyme II accounted for 5-10% of the total enzyme activity in extracts of unadapted cells, in agreement with previous results8. In adapted cells, however, this enzyme was responsible for 50-70% of the total activity (Fig. 1b). Thus, although there was no detectable difference between the levels of m³A DNA glycosylase I in adapted and unadapted cells, m³A DNA glycosylase II was present at a 20-fold higher level in adapted cells. This induced activity was eluted before the constitutively expressed enzyme I on Sephadex G-75 (Fig. 1b), indicating a higher molecular weight of enzyme II.

The identity of the induced enzyme as m³A DNA glycosylase II was confirmed by experiments with an E. coli tagA mutant (BK2114), which lacks enzyme I. In this strain, the level of m³A DNA glycosylase activity was low, but could be induced 20- to 40-fold by exposure to MNNG (Table 1). The induced activity was resistant to inhibition by free m³A, as expected for enzyme II, and also exhibited the heat stability of enzyme II. The separate identities of enzyme I and the inducible enzyme II is further supported by the observation of Evensen and Seeberg that E. coli alk mutants are deficient specifically in the latter enzyme11. In agreement with previous studies of the adaptive response², MNNG was a good inducer of m³A DNA glycosylase II, whereas methyl methanesulphonate was less efficient and 4-nitroquinoline-1-oxide had no effect. An E. coli strain (BS21) which expresses the adaptive response constitutively and contains high levels of the methyltransferase acting on m⁶G (ref. 12), did not contain a similarly elevated amount of m³A DNA glycosylase II, although it had slightly more (twoto threefold) enzyme than did unadapted wild-type cells.

In addition to the increased release of m^3A from alkylated DNA, extracts from adapted cells were also active on other N-methylated purines in DNA. Thus, whereas only extremely low levels of DNA glycosylase(s) which released m^3G and 7-methylguanine (m^7G) from methylated DNA were detectable in extracts of unadapted cells, such activities were easily demonstrable in crude extracts of adapted cells (Fig. 2, Table 1). Even in the latter extracts, however, m^7G DNA glycosylase activity was quite low; in assay conditions which resulted in complete release of m^3A and m^3G from methylated DNA, only $\sim 0.3\%$ of the m^7G was enzymatically released.

Several other DNA glycosylases, which specifically recognize different types of damaged bases in DNA, have been found in E. coli. A survey of the levels of these activities in extracts from unadapted and adapted cells showed that only m³A DNA glycosylase II, m³G DNA glycosylase and m³G DNA glycosylase activities were induced during adaptation by MNNG treatment (Table 1). Note that the amount of formamidopyrimidine DNA glycosylase was not increased, although this enzyme acts on a major secondary alkylation product¹³.

The inducible DNA glycosylase activities for m³A, m³G and m⁷G appear to be associated with a single enzyme as the relative levels of induction of the three activities were the same (10-to 40-fold) in different experiments, although the small amounts of m³G and m⁷G DNA glycosylase activity in unadapted cells made accurate measurements difficult. (Neither activity was as efficiently induced as the methyltransferase for m⁶G [ref. 14].) Furthermore, we have been unable to separate the inducible DNA glycosylase activities by a variety of chromatographic procedures (DEAE-cellulose, Sephadex gel chromatography, DNA-cellulose).

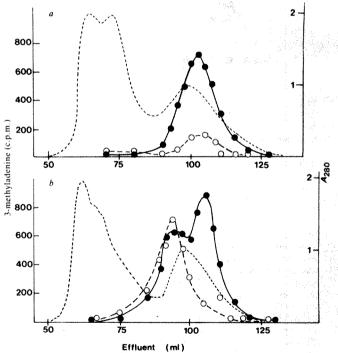


Fig. 1 3-Methyladenine DNA glycosylases I and II in E. coli B/r before and after exposure to MNNG. Cells were adapted by growth in MNNG (1 µg ml⁻¹) for 60 min. Extracts from 3 g cells were prepared by grinding with sand and partially purified by streptomycin and ammonium sulphate fractionation9. Partially purified extracts were chromatographed on Sephadex G-75 (1 cm diameter × 100 cm) in 0.3 M NaCl. 0.05 M Tris-HCl pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 5% glycerol. Duplicate 10-µl aliquots of each fraction were assayed in 100 µl standard assay mixture containing H-dimethylsulphate-treated DNA (5,000 c.p.m.), with or without product inhibition of m³A DNA glycosylase I by free m³A (3mM). a, Unadapted cells; b, adapted cells. A_{280} ; \bullet , assay without free m³A; \circ , assay in the presence of 3 mM m³A.

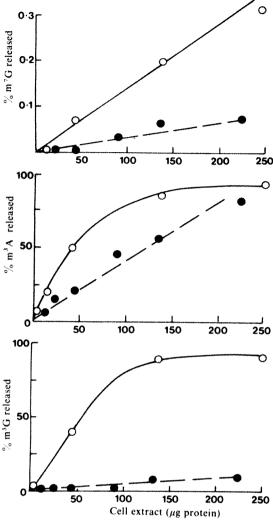


Fig. 2 DNA glycosylase activities for methylated purines in extracts of unadapted and adapted E. coli. Cultures (10 ml) of E. coli B/r were adapted by growth in MNNG (1 μ g ml⁻¹) for 60 min. Cells were collected by centrifugation, washed and disrupted by sonication in 0.07 M Tris-HCl pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol. Debris were removed by centrifugation (10,000 g, 5 min) and the crude extract used to assay the release of methylated purines. The assay mixture (100 µl) contained: 0.07 M HEPES-KOH pH 7.8, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 2 μ g ³H-dimethylsulphate-treated M. luteus DNA (50,000 c.p.m.) and 0-250 µg extract protein. After 20 min at 37 °C, mixtures were cooled to 0 °C, precipitated with 2 volumes of ethanol and centrifuged (10 min, 10,000 g). Supernatants were concentrated by evaporation to ~30 µl, supplemented with authentic methylated purines and analysed by HPLC using a Varian model 5000 chromatograph. Separation was on a Whatman Partisil 10/25 SCX column using a gradient of ammonium formate, pH 4.0, in 8% methanol essentially as described in ref. 21. The identities of released methylpurines were confirmed by paper chromatography on Whatman 3MM paper. Chromatograms were developed in methanol/ethanol/HCl/H2O (50:25:6:19) for 24 h. Radioactivity associated with the positions of authentic marker compounds was determined as described previously8. •, Unadapted cells; O, adapted cells.

Support for this conclusion comes independently from Thomas et al. 10, who partly purified enzyme II from unadapted E. coli. They found that the three DNA glycosylase activities were associated with a protein of molecular weight 27,000, and the m³A- and m⁷G-releasing activities were also shown to exhibit identical rates of heat inactivation, and to co-migrate on isoelectric focusing. The m⁷G DNA glycosylase associated with enzyme II probably accounts for the low level of this activity previously reported in E. coli¹³

Table 2 Excision of methylated purines from the DNA of dimethylsulphate-treated E. coli

Incubation time after alkylation (min)		Alkylated bases remaining in DNA (pmol per 10 ¹¹ cells)						
		m^7G	m^3A^*	m^3G	m^7A	m^1A		
Unadapted	$\begin{cases} 0\\30 \end{cases}$	124.7 106.7	0.93 0.92	1.61 0.78	4.15 2.55	0.34 0.18		
Adapted	$\begin{cases} 0\\30 \end{cases}$	114.5 109.1	0.94 0.54	0.68 <0.03	3.20 2.10	0.38 0.11		

Cultures of E. coli B/r (11) in M9 medium containing glucose (0.4%) and casaminoacids (0.1%) were adapted as described in Table 1 legend. Unadapted cultures, grown in parallel, did not receive MNNG. Adapted and unadapted cells were collected by centrifugation, washed and resuspended in unsupplemented M9 (prewarmed to 37 °C) at a concentration of 4×10^{10} cells per ml. To both suspensions was added ³H-dimethylsulphate (670 c.p.m. pmol⁻¹) to give a final concentration of 0.17 mM. After 10 min incubation at 37 °C, one-half of each suspension was diluted with 5 volumes of crushed ice and the cells collected by centrifugation. The remaining cells were diluted into full growth medium and incubated for a further 30 min before collection. DNA was extracted from cells as described in ref. 8, hydrolysed and alkylated purines analysed by HPLC as described in Fig. 2 legend.

More than 95% of the m³A initially formed was removed during the treatment with the alkylating agent8 and was not detected in the 0 min

samples.

As the potentially lethal lesion m³A is rapidly removed by the constitutively expressed enzyme I, it is unclear why an additional, inducible activity should be necessary. Further, the major alkylation lesion m⁷G appears to be an innocuous form of damage, which usually persists for a long time in both unadapted and adapted cells, although the latter have slightly better capacity to remove this base (Fig. 2). Like m³A, the minor DNA methylation product, m³G (ref. 16), projects its methyl group into the narrow groove of the DNA double helix which, unlike the wide groove, is normally free of methyl groups. In contrast to m³A, however, it is not efficiently removed in unadapted cells¹⁷. We have investigated the effect of adaptation on the rate of removal of this alkylation product in vivo. As shown in Table 2, m³G was removed quite slowly in unadapted cells, with 50% remaining in DNA 30 min after treatment. In adapted cells, however, about one-half was removed immediately during the brief treatment with an alkylating agent, and by 30 min no residual m³G was detectable. m³A was excised with a similar high efficiency in unadapted and adapted cells, whereas most of the m⁷G remained in the DNA in both cases. The minor alkylation products 7-methyladenine and 1-methyladenine, which were also resolved during HPLC of DNA hydrolysates, were inefficiently removed in adapted as well as unadapted cells (Table 2).

m³G only accounts for about 1% of the total DNA lesions after treatment of cells with methylating agents, and is thus produced at a 15-fold lower level than m³A. Since the latter, potentially lethal, lesion is rapidly removed by a constitutively expressed repair enzyme, similar amounts of m³A and m³G remain in DNA shortly after exposure. These lesions, both of which presumably can block the narrow groove of the double helix, would be expected to be of similar lethality. Consequently, the ability to repair m³G in DNA would seem to be of great advantage for cells exposed to alkylating agents. As shown here, E. coli has a low level of such a repair activity, which can be augmented following exposure to alkylating agents. A m³G DNA glycosylase activity has previously been observed in crude cell extracts of (unadapted) Micrococcus luteus 18 and human lymphoblasts 19. As m3G is removed from DNA less efficiently than m³A not only in unadapted E. coli but also in mammalian cells^{20,21}, it seems likely that m³G will generally be responsible for a major part of the cytotoxic effect of methylating agents.

We thank S. Stevens for valuable technical assistance at the MRC Cell Mutation Unit, University of Sussex, where part of this work was carried out, also Drs E. Seeberg and B. Sedgwick for bacterial strains, and Dr D. A. Goldthwait for a preprint of his paper. This work was supported in part by the Swedish MRC.

Received 23 December 1981, accepted 24 February 1982

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Adaptation to alkylation resistance involves the induction of a DNA glycosylase

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Cells exposed to low doses of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or methyl methanesulphonate (MMS) acquire resistance to both the mutagenic and lethal effects of a challenging dose of the same agents (ref. 1, and ref. 2 for a review). This response, termed adaptation, has been ascribed to the induced synthesis and accumulation of *O*-6-methyl-guanine (m⁶G) DNA methyltransferase^{3,4} which rapidly demethylates the m⁶G residues induced by the challenging dose³⁻⁵. Mutant studies, however, indicate that mutagenic adaptation and killing adaptation are at least partly under different genetic control and may therefore involve the induction of different repair enzymes^{6,7}. Whereas mutagenic adaptation correlates with the induction of the transferase, the data presented here show that killing adaptation can be ascribed to the induction of a DNA glycosylase. This inducible glycosylase releases the alkylation product 3-methyladenine (m³A) from DNA in vitro as does the constitutive m³A DNA glycosylase previously characterized by Riazuddin and Lindahl⁸. However, the enzymes are encoded by different genes and appear to have different roles in DNA repair in vivo.

We have previously isolated from MNNG-mutagenized clones of Escherichia coli a mutant that is extremely sensitive to MMS, unable to reactivate alkylated phages and deficient in m³A DNA glycosylase activity⁹. Further characterization of this strain (BK2106) has revealed that it carries two mutations, tag and ada, and not only lacks the glycosylase, but also is deficient in the induction of m⁶G DNA methyltransferase. Elimination of the ada mutation in this strain by P1 transduction renders the cell wild-type resistant to the lethal effects of MMS in agar medium (Table 1). However, the ada+ tag transductant (BK2114) is still unable to reactivate alkylated phages (Fig. 1) and shows considerable sensitivity to MMS exposure in buffer (Fig. 2). These observations suggested that E. coli may have

two pathways for the repair of m³A residues in DNA, one that is constitutively expressed and controlled by tag, and another that is inducible and controlled by ada. The inducible repair pathway would be turned on when the cells were exposed to alkylating agents in complete medium, but not when the cells were infected with alkylated phages. Table 1 shows that the adaptive treatment used to induce m⁶G DNA methyltransferase also induces high levels of m³A DNA glycosylase activity in the tag mutant (BK2114). Such induction is not observed in the tag ada double mutant (BK2106). Adapted BK2114 has also regained essentially normal ability to reactivate alkylated phages which implies that the inducible enzyme can replace the constitutive enzyme also in repair of phage DNA (Fig. 1). These results show that adaptation induces two different enzymes for repair of alkylated DNA, m⁶G DNA methyltransferase and m³A DNA glycosylase, and that both enzymes are under ada

Whereas constitutive m³A DNA glycosylase (TagI) in wildtype cells is inhibited by m³A (ref. 8), the inducible glycosylase (TagII) in BK2114 is unaffected by m³A (Table 1). Adaptation of wild-type cells also results in the induction of m³A DNA glycosylase activity not inhibited by m³A, indicating that the inducible enzyme in BK2114 is not a mutated form of the normal tag gene product, but is indeed a separate enzyme. Karran et al.9 previously observed a minor m3A-noninhibited m³A DNA glycosylase in both wild-type cells and strain BK2106. This enzyme is more heat-stable than TagI and constitutes ~5% of the total m³A DNA glycosylase activity in wild-type cells. It is probably the same as that which is induced to high levels in BK2114 by the adaptive treatment. In fact, Lindahl and co-workers¹⁰ have recently observed that the heatstable activity of m3A DNA glycosylase increases several-fold

in wild-type cells during adaptation.

The observation that the ada mutation prevents induction of two different repair enzymes suggests that the ada gene is a control gene for the adaptive response and that structural genes for one or both of the enzymes may be found elsewhere on the chromosome. While searching for structural gene mutants we observed that the alk-1 mutant^{11,12} was deficient in the induction of TagII, but proficient in induction of the transferase (Table 1). Similar results were obtained with another alk mutant (isolated by B. Duncan). No other enzyme defect has been linked to mutations in the alk gene. alk mutants are very sensitive to MMS exposure and it seems likely that the MMS sensitivity is caused by TagII deficiency, in which case the low levels of TagII activity present in wild-type cells must be essential for some repair not effected by TagI. It is possible

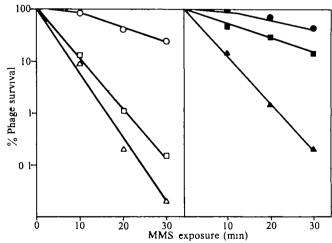


Fig. 1 Host cell reactivation of alkylated phages in non-treated and adapted cells. Phage & cI857 were exposed to 0.05 M MMS for the times indicated and plated on exponentially growing untreated (open symbols) or adapted (closed symbols) cells of AB1157 (wild type, circles), BK2106 (tag ada, triangles) or BK2114 (tag, squares). Cells were adapted as described in Table 1 legend and conditions for the plating procedure were as published previously

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Table 1 Properties of E. coli mutants defective in alkylation repair

*		Plating efficiency on nutrient	m ³ A cell ext	m ⁶ G DNA methyltransferase activity in extracts		
Bacterial strains	Relevant genotype	agar containing 3 mM MMS* (%)	Noninduced (TagI)	Induced	Induced + 3 mM m ³ A (TagII)	from induced cells (% m ⁶ G remaining in substrate)
AB1157	Wild type	97	26.5	41.2	36.3	9.5
BK2106	tag ada	5×10^{-4}	6.4	0.7	1.8	94.5
BK2114	tag	67	3.8	26.3	34.0	6.5
BK2110	ada	1.4	24.3	23.1	5.3	94.0
MS23	alk-1	0.06	25.7	23.1	1.8	6.7
BD793	$\Delta alk(mgl-attP2H)$	0.16	27.0	26.2	0.5	2.9

Strain BK2106 is a MNNG-mutagenized derivative of AB1157 (ref. 9) and carries two mutations which both contribute to its MMS sensitivity. The mutation which maps at 47 min and co-transduces with gyrA is shown here to be in the ada gene and not in the tag gene as was previously assumed. Other ada mutations have also recently been mapped to the same position on the map¹³. The tag mutation responsible for m³A DNA glycosylase (Tagl) deficiency in BK2106 has now been mapped to a region between 70 and 74 min on the chromosome (G.E. and E.S., in preparation). Strains BK2106 and BK2114 (BK2106 gyrA ada⁺); BK2110 (AB1157 gyrA ada) and AB1157; and MS23 and AB1157 (refs 10, 11) are all isogeneic pairs constructed by P1 transduction. BD793 was isolated by B. Duncan and is a his⁻ eductant of phage P2-lysogenized W1485F⁻. Cell extracts were prepared by a combination of plasmolysis and lysozyme digestion as previously described¹⁴. m³A DNA glycosylase activity was assayed by the method of Riazuddin and Lindahl⁸. The numbers presented are calculated on the basis of ethanol-soluble radioactivity released from ³H-dimethylsulphate-treated DNA using various amounts of extracts ranging from 0 to 20 µg protein. In this range a linear response was obtained between glycosylase activity and extract added. Parallel assays were also run where the reaction mixtures were subjected to polyetheneimmine TLC together with authentic 7-methylguanine and 3-methyladenine. In all cases where also run where the reaction mixtures were subjected to polyetheneimmine TLC together with radioactivity released was identified as m³A. m⁶G DNA methyltransferase activity was assayed by the method of Karran et al.³ and 20 µg protein were added to each assay. Cells were induced by growth in M9 salts supplemented with 1% glucose and 1% casaminoacids in the presence of 0.5 µg MNNG for 90 min.

* Stationary phase cells calculated relative to plating on nutrient agar without MMS.

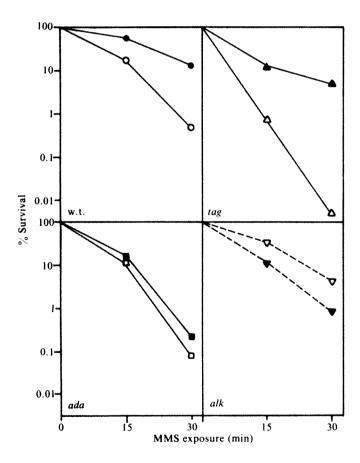


Fig. 2 Killing adaptation to alkylation resistance in wild-type and TagII-deficient mutants. Fresh overnight cultures were diluted 10-fold in K medium (M9 salts+1% glucose and 1% casaminoacids). To one-half of each culture was added MNNG at a final concentration of $0.5 \mu g \, ml^{-1}$ while the other half served as an untreated control. The cells were grown for 90 min at 37 °C and then washed once by centrifugation and resuspended in 1/10 volume of phosphate buffer. MMS was added at a final concentration $0.06 \, M$ for strains AB1157 (wild type; \bigcirc , \bigcirc), BK2114 (tag; \triangle , \triangle) and BK2110 (ada; \square , \square), and of $0.02 \, M$ for MS23 (alk-1; ∇ , \blacksquare). At intervals, samples were plated for survival after appropriate dilution. Closed symbols refer to adapted cell cultures (MNNG-treated) while open symbols represent untreated controls.

that TagII has a broader substrate specificity than TagI and is responsible for repair of a quantitatively minor but biologically important alkylation product other than m^3A . TagI has a narrow substrate specificity and a very low K_m value for its substrate, suggesting that it will quickly bind and remove m^3A from alkylated DNA. Other minor products, however, could perhaps be removed by TagII. The alk mutants seem to have less residual TagII activity than the ada mutant, which would explain the greater sensitivity of the former compared with the latter (Table 1).

Mutants defective in the adaptive response were first described by Jeggo⁷. They were selected on the basis of their deficiency in mutagenic adaptation but most of them also appeared to be defective in killing adaptation. One mutant, however, was only defective in mutagenic adaptation. Jeggo et al.6 have further reported that polA mutants defective in DNA polymerase I are defective in killing adaptation, but show normal mutagenic adaptation. These studies are consistent with the view that mutagenic adaptation and killing adaptation are due to the induction of separate repair pathways. They support the view that m⁶G DNA methyltransferase is responsible for mutagenic adaptation since transferase repair of m⁶G will not require DNA polymerase I. The ada+-dependent inducible glycosylase may well be responsible for killing adaptation because DNA polymerase I is needed to complete repair of the apurinic site formed by the glycosylase action. A critical test for this interpretation is that alk as well as ada mutants should be defective in killing adaptation. Figure 2 shows that this is indeed the case and, furthermore, that the alk-1 mutant is even sensitized by the adaptive treatment. These results therefore strongly support the view that TagII, not the transferase, is responsible for killing adaptation to alkylation resistance.

Thus E. coli seems to have two distinct m³A DNA glycosylase activities: one that is constitutively expressed and controlled by tag, and a second that is inducible and controlled by ada and alk. The constitutive enzyme appears responsible for rapid repair of m³A alkylation products in unadapted cells. The inducible enzyme seems to be required for killing adaptation to alkylation resistance and probably also for repair of some potentially lethal lesions not recognized by the constitutive enzyme in unadapted cells.

We thank Tomas Lindahl for communicating that strain BK2106 is defective in m⁶G DNA methyltransferase and for the gift of transferase substrate, Bruce Duncan and Mutsuo Sekiguchi for the gift of bacterial strains, Neil Clarke for valuable discussions, and Peter Strike for a critical reading of the

manuscript. This research was supported by the Norwegian Council for Scientific and Industrial Research.

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Binding of ATP to tubulin

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Microtubules are present in all eukarvotic cells and participate in a variety of cellular processes1. From recent studies it has become clear that nucleotides have a central role in the assembly of tubulin into microtubules²⁻⁵. The tubulin dimer binds two moles of guanine nucleotide: one (at the E-site) which can exchange with exogenous nucleotide and one (at the N-site) which does not exchange⁶. E-site GTP is hydrolysed during the assembly process^{4,7,8}. Microtubule assembly can be induced by ATP through a contaminating nucleoside diphosphokinase activity that regenerates GTP following its hydrolysis—this is associated with tubulin polymerization^{4,9,10}. Recent reports suggest that ATP has other effects on the assembly kinetics^{11–13}, and we have shown 14,15, using tubulin preparations lacking the nucleoside diphosphokinase activity, that ATP may play a critical part in the regulation of microtubule formation by binding to tubulin at a site which is distinct from the N- and E-sites. Kinetic data suggest a model in which ATP can act at the level of nucleation to stimulate assembly 15. We now report the first direct evidence for the binding of ATP to tubulin and show that the corresponding dissociation constant is in a range of physiological significance.

In attempting to demonstrate the existence of a tubulin-ATP complex and to determine the dissociation constant, we found that equilibrium dialysis and centrifugation methods were unsuitable because they were too slow for such an unstable protein and because of the relatively weak binding of the nucleotide. We therefore chose the method of Hummel and Dreyer¹⁶ which is rapid and maintains the protein-ligand complex in equilibrium conditions. It has been used previously to determine guanine nucleotide binding to tubulin^{17,18}. In this method the protein is placed in solution with a given amount of ligand, and then applied to a gel filtration column which is equilibrated with the same concentration of ligand. As the protein emerges from the column at the excluded volume, it bears extra ligand above the equilibrium level, while at the included volume, the ligand level is depressed by the amount of ligand removed by the protein. The amount of ligand above the baseline level is equivalent to the protein-ligand complex and can be applied to the equilibrium expression, together with the concentration of free ligand, to calculate the dissociation

Tubulin was prepared by chromatography on DEAEcellulose (Whatman DE-52) as described elsewhere⁶. It has been shown to be more than 99% homogeneous electrophoretically and to have only minimal nucleoside diphosphokinase activity15. Figure 1 shows a representative profile obtained by passage of tubulin down a Sephadex G-25 column equilibrated with ³²P-ATP. As the protein eluted from the column, there was a corresponding increase in the level of nucleotide above

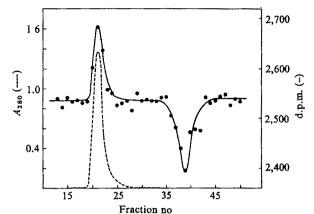


Fig. 1 Example of a Hummel-Dreyer column used to measure ligand binding. A Sephadex G-25 column (25×1 cm) was equilibrated with PM buffer (10 mM phosphate, 5 mM MgCl₂, pH 7.0) containing 20 μ M [γ - 32 P]ATP. Tubulin (\sim 10 mg ml $^{-1}$) was prepared as described elsewhere 5,10 , dialysed extensively against PM buffer, centrifuged at 140,000g for 30 min, then mixed with an equal volume of PM buffer with $40 \mu \text{M} \left[\gamma^{-32}\text{P}\right]\text{ATP}$; 0.6 ml was applied to the column followed by elution with column buffer and 0.5-ml fractions were collected. Fractions were scanned for protein at 280 nm and the radioactivity determined by spotting aliquots on glass fibre filters (Whatman GF/C) and counting these using a Beckman LS7500 liquid scintillation counter. All chromatography was performed at room temperature.

the equilibrium value followed by a trough at the included volume. We ran five such columns for a series of ligand concentrations from 10 to 100 µM. Two peak fractions from each column were used to determine the level of ligand binding. The results are presented in Table 1.

The dissociation constant was calculated based on a stoichiometry of one binding site per tubulin dimer. The relative agreement in the values of K_d obtained at various ligand concentrations supports this assumption. The stoichiometry is usually determined from the amount of ligand bound in saturating conditions. However, with such a low binding affinity, the background levels of ligand would need to be so high in order to saturate the binding site that no reasonable precision could be obtained. Moreover, as relatively weak binding results in a rather modest difference between the peak of bound ATP and a substantial baseline, even at relatively low ATP concentrations, the average value calculated for the dissociation constant, 2.3×10^{-4} M, is only approximate. Clearly, however, ATP does

Table 1 Binding of ATP to tubulin measured by the Hummel-Dreyer

Column ATP (µM)	Protein- bound ATP (µM)	Peak protein (μΜ)	mol ATP/mol protein	<i>K</i> _d (×10 ⁴)
10	1.06	12.2	0.087	1.1
	0.665	7.4	0.090	1.0
20	1.17	12.8	0.091	2.0
	0.804	8.4	0.096	1.9
25	2.02	15.9	0.13	1.7
	1.20	10.2	0.12	1.8
50	2.10	10.0	0.21	1.9
	2.25	11.1	0.20	2.0
100	2.06	15.1	0.14	6.1
	1.87	8.5	0.22	3.5

Gel filtration columns equilibrated with the indicated concentration ²P]ATP were run as described in Fig. 1 legend. The radioactivity in the two peak fractions was determined by liquid scintillation counting and the protein concentration by the method of Lowry et al.20. Kd was determined from the equilibrium expression:

 $K_d = [tubulin][ATP]/[tubulin - ATP].$

The stoichiometry was assumed to be one binding site per tubulin dimer (molecular weight 100,000).

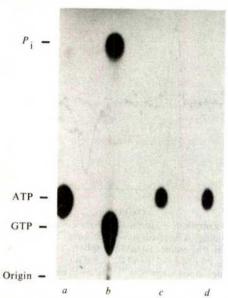


Fig. 2 Test for ATP hydrolysis following microtubule assembly. (~40 µm Tubulin (\sim 40 μ m in assembly buffer (10 mM 2-(N-morpholino)ethanesulphonic acid, 2.0 mM Mg²⁺, 1.0 mM EGTA, 3.4 M glycerol, pH 6.4) with 1.0 mM GTP and 0.2 mM [γ - 32 P]ATP was warmed to 37 °C to allow microtubule polymerization. In these conditions, ATP significantly enhances the assembly kinetics15. Monitoring of the turbidity at 350 nm indicated that a steady state was reached within 10 min. Aliquots taken at 0 and 10 min were mixed with an equal volume of 95% ethanol, cooled to 0 °C and centrifuged to remove the precipitated protein. These were spotted on to a PEI-cellulose thin-layer plate (Brinkman), then the plate was washed by immersion in 95% ethanol and dried. Ascending chromatography was performed using 1.0 M LiCl as the solvent, followed by autoradiography using Kodak X-Omat X-ray film. a, ATP standard; b, GTP and P, standard; c, assembly sample (0 min); d, assembly sample (10 min).

bind to tubulin and the calculated K_d is within an order of magnitude of cellular ATP levels, that is, ~2 mM (ref. 19). It is therefore reasonable to suggest that ATP regulates microtubule formation in vivo according to changes in its distribution and concentration.

As ATP is bound to tubulin and affects the rate of microtubule formation it is appropriate to ask whether the ATP supplies energy through hydrolysis. Therefore, microtubules were assembled in the presence of $[\gamma^{-32}P]ATP$ and the solution was analysed by TLC for the presence of free phosphate. Although microtubule formation had reached a steady state, no free phosphate was observed (Fig. 2). (The appearance of a minor amount of GTP in the autoradiogram was due to a trace of residual nucleoside diphosphokinase activity in the tubulin preparation.) To exclude the possibility that the 32P was incorporated into protein, the proteins in a similar microtubule mixture were resolved by electrophoresis and autoradiographed; no 32P incorporation was detected. As ATP is hydrolysed neither to produce inorganic phosphate (Pi) nor to phosphorylate a protein during microtubule formation, the effect of ATP on assembly must involve some other mechanism, perhaps allostery

This work was supported by research grants GMS 20338, EHS P30 ESO1896 and GMS 7232 from the NIH and the Agricultural Research Station.

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Collective variable description of small-amplitude conformational fluctuations in a globular protein

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Globular proteins in the native state undergo small-amplitude conformational fluctuations1, which can be observed experimentally, for example, as temperature factors in X-ray crystal--s or through their effects on the relaxation times in NMR6. The fluctuations can also be observed as the trajectories of each atom of a protein molecule generated by the theoretical method of molecular dynamics^{5,7,8}. These studies indicate that atoms or groups of atoms in the molecule, especially those near the surface, are in a 'fluid-like' state, which means that the forces acting on them have an impulsive character and the temperature factors obtained from X-ray crystallography have an anharmonic temperature dependence. More work is clearly needed to characterize better the state of the protein interior. Thus we have now calculated the shape of the conformational energy surface of a small protein, basic pancreatic trypsin inhibitor (BPTI), near its minimum. We find that for most degrees of freedom corresponding to collective conformational changes, the protein molecule behaves harmonically or like a solid.

In thermal equilibrium a protein molecule takes on a microscopic conformation with a probability determined by the con-

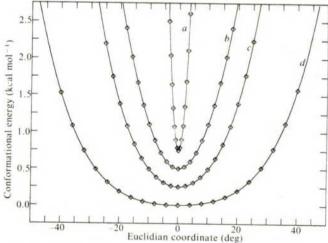
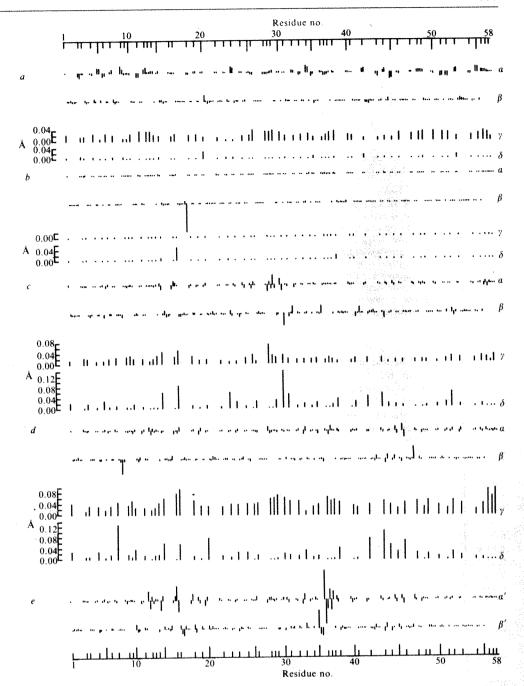


Fig. 1 Conformational energy curves calculated for actual conformational changes induced by variations in four variables, variables 37 (a), 118 (b), 179 (c) and 235 (d). The abscissa is the coordinate measured by euclidian distance defined as $d = \{\sum_{i=1}^{241} (\Delta \theta_i)^2\}^{1/2}$, where θ_i is a common notation denoting either ϕ or ψ or χ , and $\Delta\theta_i$ is the current value of θ_i minus that of θ_i at the energy minimum. Conformational energy values for a, b and c are off-set by 0.75, 0.50 and 0.25 kcal mol⁻¹ respectively for c are off-set by 0.75, 0.50 and 0.25 kcal mol respectively for clarity. \diamondsuit , Calculated conformational energy. —, Best-fit curves of the form of $B\xi^2 + C\xi^3 + D\xi^4$. Best-fit values of $B(\text{kcal-mol}^{-1} \text{deg}^{-2})$, $C(\text{kcal mol}^{-1} \text{deg}^{-3})$ and $D(\text{kcal mol}^{-1} \text{deg}^{-4})$ for the four curves are: a, 0.15, 0.1×10⁻², 0.1×10⁻³; b, 0.0061, 0.3×10⁻⁷, -0.6×10⁻⁶; c, 0.0023, 0.2×10⁻⁵, 0.1×10⁻⁵; and d, 0.00032, 0.3×10⁻⁶, 0.4×10⁻⁶. The first three curves are essentially parabolas in the range of thermal fluctuations. A pharmonic tially parabolas in the range of thermal fluctuations. Anharmonicity is observed in the last curve.

Fig. 2 Make-up of four eigenvectors of the second-derivative matrix, numbers 37 (a), 118 (b), 179 (c) and 235 (d), and equilibrium correlations of fluctuations in dihedral angles (e). In a-d, the value of the jth component, uin of the ith eigenvector is represented by a bar (upwards when positive, downwards when negative) against serial number j of variable dihedral angles. When j refers to a main-chain angle, the bar is shown in lines α ; when to side-chain angle, in lines β . Dihedral angles in the kth residue are arranged in the order ϕ_k , χ_k^1 , numbers, Residue $\chi_k^{\ell}, \ldots, \psi_k$. instead of the serial numbers of dihedral angles, are given on the abscissa. The displacements of atoms in collective conformational changes caused by changes in the collective variables are shown in lines y and δ : γ , displacements of C^{α} atoms, and δ , average displacements of sidechain atoms relative to a local framework fixed to main-chain atoms in the same residue. The magnitude of the collective conformational change is taken as the root mean square thermal deviation of collective variable ξ_i calculated from the actual energy curve shown in Fig. 1. e, Example of relative equilibrium correlations of fluctuations in dihedral angles defined by $\langle \Delta \theta_i \Delta \psi_{35} \rangle / \langle (\Delta \theta_i)^2 \rangle^{1/2} \langle (\Delta \psi_{35})^2 \rangle^{1/2} \rangle$. α' and β' refer to main-chain and side-chain dihedral angles. Residue 35 is located in an extended strand, which is roughly sandwiched between two other strands; it touches residue 11 in one strand and residue 17 in the other.



formational energy according to the Boltzmann law. The distribution of various fluctuating conformations can therefore be clarified by elucidating the shape of the conformational energy surface near the minimum point corresponding to the most stable conformation. Very near to the minimum, the energy surface is a multidimensional parabola characterized by the second-derivative matrix at the minimum.

The multidimensional parabola is most easily described in terms of a set of collective variables, $\{\xi_i\}$, corresponding to the eigenvectors of the second-derivative matrix

$$\xi_i = \sum_i u_{ij} \Delta \theta_i \tag{1}$$

where u_{ij} is the jth component of the ith eigenvector and $\Delta\theta_i$ is the deviation of the jth internal variable from that at the minimum point. A change in the variable ξ_i causes simultaneous and correlated displacements of many atoms in the molecule. Thus $\{\xi_i\}$ s are predicted to be natural variables describing conformational fluctuations in which strong correlations are known to exist among atomic displacements.

In terms of the collective variables, the conformational

energy, E, near the minimum point is given by

$$E = \frac{1}{2} \sum \lambda_i \xi_i^2 \tag{2}$$

where λ_i is the *i*th eigenvalue of the second-derivative matrix. For the range in which equation (2) applies, the collective conformational changes caused by changes in each ξ_i are mutually independent (that is, not correlated), and any conformational change can be predicted as superpositions of changes in ξ_i .

If the conformational energy can be expressed by equation (2), the conformational fluctuations will be harmonic or solid-like. Our previous study using a Monte Carlo method suggested that for many collective variables, the conformational energy was parabolic in the range of thermal fluctuations. Now we have calculated the second-derivative matrix at the minimum energy. Energy curves are calculated for a thermal excitation range of 0.3 kcal mol⁻¹ for actual conformational deformations caused by changes in each of the collective variables.

As in our earlier work⁹, bond lengths and bond angles in the protein are fixed and only dihedral angles of a main chain $\{\phi, \psi\}$ and side chains $\{\chi\}$ are treated as variables. The conformational

Table 1 Classification of anharmonic energy curves

		No. of anharmonic collective variables Type					
Rank order of collective variables	Range of eigenvalues (kcal mol ⁻¹ deg ⁻²)	\ ' /	/ n /	\ III /	\	y	Total
1- 53	$1.30 \times 10^{4} - 1.00 \times 10^{-1}$		\bigcirc		\circ		0
54-160	$0.99 \times 10^{-1} - 0.61 \times 10^{-2}$	3	1				4
161–180	$0.60 \times 10^{-2} - 0.45 \times 10^{-2}$				1		1
181–206	$0.44 \times 10^{-2} - 0.30 \times 10^{-2}$	2	6		1		9
207–229	$0.29 \times 10^{-2} - 0.10 \times 10^{-2}$	1	14	3			18
230–241	$0.99 \times 10^{-3} - 0.20 \times 10^{-3}$	1	3	5		2	11
Total		7	24	8	2	2	43

The energy curves for all the collective conformational changes are well fitted by fourth-order curves of the form $B\xi^2 + C\xi^3 + D\xi^4$, except for four curves classified as types IV and V. A curve is harmonic when the terms $C\xi^3$ and $D\xi^4$ are insignificant in the range of thermal fluctuations. As a measure of the range of thermal fluctuations, we take $[-\xi_0, \xi_0]$, where ξ_0 is defined by $\frac{1}{2}B\xi_0^2 = 0.3$ kcal mol⁻¹. A curve is anharmonic if $|C\xi_0^4/B\xi_0^2| \ge 0.1$ and/or $(D\xi_0^4/B\xi_0^2) \ge 0.1$, and may be one of five types according to its shape. Type I: curves where $|C\xi_0^3/B\xi_0^2| \ge 0.1$ and $(D\xi_0^4/B\xi_0^2) \ge 0.1$. In conformational changes caused by variations in these collective variables, large changes in dihedral angles and atom positions are confined to one or two side chains. If curves where $1.0 > (D\xi_0^4/B\xi_0^2) \ge 0.1$. III: curves where $(D\xi_0^4/B\xi_0^2) \ge 1.0$. Conformational changes caused by variations in collective variables of types II and III are not localized in any part of the molecule. 4 of 24 type II curves and 5 of 8 entries in type III curves have non-negligible third-order terms, that is, $|C\xi_0^3/B\xi_0^2| \ge 0.1$. IV: conformational changes are localized in one or two side chains with smaller changes elsewhere. A peak of the energy curve is due to torsional energy for dihedral angle(s) of the side chain(s). V: Almost free rotation of a side chain (lysine) dihedral angle. Peaks are due to the non-bonded energy.

space therefore consists of 241 variables (116 main-chain and 125 side-chain dihedral angles). Hydrogen atoms are not treated individually but are included in calculations for the atom to which they are bonded, except for those expected to participate in hydrogen bonding. Geometrical parameters and energy functions are those used previously9, except for the energy functions for hydrogen bonding; energy functions of the form of (Ar⁻¹²+Br⁻¹⁰) are used. Solvation effects are not considered, that is, the molecule is treated as being in vacuo.

By starting from the conformation with the least energy in the previous Monte Carlo study, the conformational energy can be minimized in the 241-dimensional conformational space by the Newton method¹⁰ using first and second derivatives. The minimization is continued until a conformation is found in which all components of the first derivative are <0.02 kcal mol⁻¹ deg⁻ and all eigenvalues of the second-derivative matrix are positive.

Figure 1 shows representative energy curves calculated for actual collective conformational changes. Serial numbers are given to the collective variables, corresponding to collective conformational changes in the descending order of the magnitude of the eigenvalues. For 198 of 241 collective conformational changes, the energy curves agree well with the parabola derived from the second-derivative matrix by the criterion described in Table 1 legend.

Deviations from the parabolas are observed in curves for the remaining 43 collective variables. All but five of them are members of the 61 softest variables (variables 181-241; see Table 1). We classified the 43 anharmonic collective variables into five types; 31 are such that the fourth-order terms have non-negligible positive contributions in the thermal fluctuations (see, for example, curve d in Fig. 1).

Thus, for many (~80%) of the collective conformational changes the conformational energy is harmonic. This strongly supports the use of collective variables to describe conformational fluctuations.

The nature of the conformational change induced by variation in the collective variable, ξ_o is defined by the components, u_{ip} of the eigenvector. The make-up of typical eigenvectors is shown in Fig. 2. Due to the complexity of the protein structure, there are many types of collective variable, most of which express non-local conformational changes (Fig. 2a, d). Whereas in variable 118, changes in dihedral angles and atom positions are localized mainly in one surface side chain (Fig. 2b), in variable 179, changes are localized mainly in a flexible loop structure consisting of residues 26-30 (Fig. 2c).

To characterize the shape of the multidimensional energy surface in the range of thermal fluctuations, contributions from higher-order cross-terms must be known. Even among the

harmonic variables these may be non-negligible. Some may cause anharmonic fluctuations such as transitions between energy minima. The anharmonicity observed in a few variables and higher-order cross-terms not considered here may account for the anharmonic behaviour of the conformational fluctuations observed in X-ray temperature factors²⁻⁵ and molecular dynamics5,7,8

There are two difficulties in our approach. First, the constraints on bond angles increased the energy barrier separating energy minima8. If the bond angles were to relax adiabatically, the conformational energy surface might be softer than we have allowed here, in which case the number of harmonic collective variables might be an over-estimate. Second, by neglecting the effects of solvent molecules, some changes in the surface of the molecule may not have been considered. However, our qualitative conclusion, that conformational fluctuations are largely harmonic and can therefore be described in terms of the collective variables, still holds. Because kinetic energy has not been considered, the collective variables defined here do not correspond to normal modes of vibration. The collective variables are useful in describing populations of conformations in equilibrium.

If we assume that, in the thermal range considered, the energy surface can be approximated in all degrees of freedom by a multidimensional parabola, we can calculate equilibrium correlations of fluctuations in dihedral angles from the secondderivative matrix (see Fig. 2e). Strong correlations exist between dihedral angles that are close in space, reflecting various collective conformational changes in the protein. If we can assume harmonicity of the energy surface, we will be able to calculate various quantities that reflect conformational fluctuations from the second-derivative matrix and to compare them with experimental results.

We thank Professor T. Ooi and Dr K. Nishikawa for providing a computer program to calculate conformational energy. Computation was done at the computer centres of Kyushu University and the Institute for Molecular Science. This work was supported by grants-in-aid from the Ministry of Education, Japan.

Received 9 November 1981, accepted 4 March 1982

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MATTERS ARISING

Pleistocene and Holocene climates in Africa

In examining the question of Pleistocene and Holocene climatic fluctuations in North-West Africa, Sarnthein et al. distinguish between hypotheses that suggest distinct latitudinal shifts and those assuming stable latitudinal positions but contraction and expansion of the arid zone, and they cite Nicholson and Flohn² as an example of the former. This is clearly incorrect. We do indicate such shift at 18,000 yr BP, but not for later periods: and we clearly show a pronounced contraction of the arid zone during the climatic optimum at 6,000 yr BP. We do suggest that both a shift in the position and changes in intensity of the major circulation features are responsible for African rainfall changes in the late Pleistocene and Holocene.

Sarnthein et al. cite primarily two lines of evidence of 'stable' latitudinal positions of circulation features. The first, concerning the location of the westerly wind belt over North-West Africa, is contradicted by recent findings3, which suggest it extended to 20-40° N at 18,000 yr BP and at present. Furthermore, the main Pleistocene displacement of that zone would probably have been during the summer and its southern most position (winter months) may not have differed much from present. The second piece of evidence is the mean position of the 'Harmattan' dust outbreaks, which are related to the midtroposphere jet. The term 'Harmattan' is misused: today it is generally understood to be the surface, northeasterly tradewind flow and not the upper-level easterlies. Furthermore, one cannot conclude, as those authors did, that the position of the ITCZ (intertropical convergence zone) is necessarily coincident with the position of the dust carried by the 'Harmattan' flow (or jet). This flow is a direct consequence of the temperature gradient between the Sahara and the relatively cooler and more humid air further south4. As long as the southern Sahara exists, and given the fixed Atlantic boundary to the south, the position of this easterly flow aloft (the mid-tropospheric jet) is relatively fixed. The ITCZ, on the other hand, is both dynamically and thermally determined and its position most directly inter-hemispheric thermal reflects differences, which did vary greatly throughout the late Pleistocene and Holocene. Although at present the ITCZ and related disturbances over West Africa coincide with the mid-tropospheric jet and Harmattan, they need not necessarily

The shear associated with the jet is critical for the formation of West African

disturbances4 and a weakened easterly flow could conceivably, as suggested by Sarnthein et al.1, have been a factor in sub-Saharan aridity ~18,000 vr BP. Indeed, this may also have been a factor in the recent Sahel drought5. However, the authors present no evidence of a stable position of other circulation features, such as the westerlies or the ITCZ, and it is highly unlikely that the latter was not displaced towards the equator in late Pleistocene, when glaciation and temperature changes were most pronounced in the Northern Hemisphere and interhemispheric thermal contrast must have been minimal.

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TETZLAFF, SARNTHEIN AND WOLTER REPLY—The comments of Nicholson give us the opportunity to clarify possible misunderstandings of our concepts and conclusions on the underlying causes of climatic change in North Africa¹. Nicholson particularly questions our evidence of 'stable' latitudinal positions of circulation features.

With regard to the extension of the westerlies, we basically agree with Nicholson, although we did not say too much about it in our article¹ and cited only indirect evidence. The westerlies indeed extended to 20–40° N over North-West Africa, in both periods considered, not only at 18,000 yr BP, but also at the present time. Nevertheless, average westerly wind vectors are substantiated on land only by proxy data² for precipitation due to synoptic disturbances in the westerlies.

In the Atlantic, the subtropical gyre and the coastal upwelling off the western Sahara essentially did not have a more southerly position around 18,000 BP and this provides additional evidence for a stable southern fringe of the westerlies during both winter and summer average seasons³. This contradicts a general equatorward displacement of the climatic belts south of the present westerlies in the Northern Hemisphere during cold stages, as suggested by Nicholson.

The other feature questioned—the relationship between the mean position of dust outbreaks and the position of the middle and lower tropospheric part of the ITCZ, in particular the monsoonal front-needs a deeper discussion of the meterological structures. Along a trajectory in the continental trades southwards, the average temperature of the lower would continuously troposphere increase^{4.5}, at ~18,000 BP as well as at present. The middle tropospheric easterly winds which carry the main Saharan dust outbreaks require the opposite horizontal gradient. This means a southward sloping isobaric plane on the southern fringe of the subtropics. Like other authors⁶, we call these easterlies 'Harmattan' for our convenience.

We again agree with Nicholson that an equatorward temperature such decrease can be produced only by the cooler tropical air masses, the northern boundary of which is called monsoonal front and coincides with the ITCZ near the surface over land at the present. Finally, the maximum slope of the isobaric surface can also result in barotropic instability providing the energy for the easterly waves. This implies that the easterly (Harmattan) winds, the lower tropospheric parts of the ITCZ (Fig. 1a in ref. 1) and the easterly waves (including the dust transport in their wake) are three different effects of the same dynamic structure and their positions are necessarily closely related. We feel that some authors5 imply a connection between the positions of the maximum rainfall zone and the northern boundary of the monsoonal air masses. They are assumed to be coupled by the downdrafts of the storms in the rainfall zone. However, recent findings5 do not confirm such a direct relationship. Of course, the historical position of the ITCZ in North Africa is averaged over a number of cyclic processes on the synoptic, annual and long-term climatic scale, but it is not necessarily coupled with interhemispheric temperature differences as postulated by Nicholson. A separate dynamic ITC (intertropical convergence) equatorwards of the monsoonal front over Africa at 18,000 yr BP is theoretically possible, but not probable. As a result we consider our conclusions1 valid. They strongly suggest that the dust-carrying winds, the Harmattan and the Trades, the subtropical anticyclone and also the ITCZ near the surface were not displaced equatorwards in western North Africa during the last glacial stage. Thus the fluctuations in the degree of sub-Saharan aridity/humidity should be related to factors such as the intensity and frequency of rain-bearing disturbances8 rather than to the average surface position of the ITCZ.

coincide.

In our article¹, we did not intend to misinterpret Nicholson and Flohn⁹ when citing their hypothesis of distinct latitudinal shifts of the subtropical climatic belt from interglacial to glacial times. Accidental over-simplification of the alternatives may be one of the reasons for the apparent wrong citation. On the other hand, we feel rather corroborated in our statement by the comments of Nicholson discussed above.

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Western diets and faecal nitrosamines

THE paper by Suzuki and Mitsuoka¹, while apparently demonstrating clear differences in nitrosamine production in individuals on different diets, makes unwarranted claims for the significance of these findings.

Japan has a low incidence rate of large bowel cancer compared with the United States² or Australia³. International correlation studies^{4,5}, studies of subcultures within societies⁶, and case-control and cohort studies of individuals⁷⁻¹⁰ have implicated dietary factors in colon carcinogenesis. Current hypotheses involve fat^{4,9,11}, fibre^{9,11}, alcohol¹¹, female sex hormones¹², vitamin A¹³, and cruciferous vegetables⁸ either as promoting colon cancer, via endogenous and bacterial bile acid metabolism, or as protective agents.

To test the hypothesis that a balanced Western diet constitutes a greater risk of exposure to nitrosamines (which act as initiating agents in some cancers) it is necessary to design an experiment which actually uses a balanced Western diet. The

diet used by Suzuki and Mitsuoka bears no resemblance to a normal Western diet. Specifically, the energy content of a typical Western diet^{14,15} is 9.7-10.5 mJ per is 9.7-10.5 mJ per day, compared with 11.8 mJ per day in the study of Suzuki and Mitsuoka. As a percentage of energy, protein intake is normally 12-15% (as opposed to 25%), fat ~40% (compared with 55%), carbohydrate ~45% (as opposed to 20%). In some European countries, certain vegetables may be consumed for breakfast but nowhere are salad vegetables eaten for breakfast. Indeed these vegetables were deliberately added to the 'typical' Western breakfast to increase nitrate intake. This ensures a good test of an hypothesis but not the one we are led to believe is being tested by the authors.

Although Suzuki and Mitsuoka have established that a very high protein and fat diet with a large intake of nitrate increases the faecal output of nitrosamines, they have not demonstrated that this has anything to do with differences existing in the real world.

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SUZUKI AND MITSUOKA REPLY—We agree with Potter that the diet used in our study does not exactly resemble a typical Western diet. To determine that nitrosamine formation in the intestine depends on diet, we used in our experiments a diet which contained a large

amount of nitrosamine precursors. Our study demonstrated that the faecal output of nitrosamines was increased by this kind of Western-style diet. However, we cannot implicate these diets directly in the aetiology of colon cancer. Further investigations of nitrosamine formation in the intestine in populations of high and low risk for colon cancer are necessary.

With respect to the p.p.b. analysis of nitrosamines in biological specimens, there are several problems associated with artefacts and contamination during the analysis. Nitrosamines are generally extracted from the specimens in alkaline conditions, because this inhibits nitrosation. We have noticed recently, however, that artificial formation of nitrosamines occurs in these conditions. This reaction seems to resemble the 'alkali effect' reported by Tozawa1. We have used 1 mM of morpholine to monitor nitrosamine formation during the analysis, as morpholine is nitrosated 500 times more readily than piperidine2. However, we have recently found that morpholine is more difficult to nitrosate than piperidine in alkaline conditions, which renders morpholine unsuitable for use as a monitor of possible artificial formation of nitrosamines. We are re-investigating the faecal output of nitrosamines, thus our data³ may require correction.

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BOOK REVIEWS

Public rights, private land

Timothy O'Riordan

ANN and Malcolm MacEwen are an amiably dedicated couple who have spent the past three years on an exhaustive labour of love. They have visited each of the ten national parks of England and Wales, interviewed countless officers and members and consulted many other informed people, all with the aim of finding out whether the national park idea is alive and well in the land. To this important task they have also brought their own impressive knowledge of conservation and planning matters, plus Malcolm MacEwen's experience as an appointed member to the Exmoor National Park Committee. During that time he fought a partially successful campaign to protect Exmoor's magnificent moorland from being ploughed up and reseeded by landowners ready to capitalize from the grants and guaranteed prices available for intensive livestock production.

Their report is an important analysis of the current state of national parks in England and Wales. Together with another recently published document, The Economy of Rural Communities in the National Parks of England and Wales (1982), written by the Tourism and Recreation Research Unit at the University of Edinburgh, it provides by far the most comprehensive and sobering statement of the state of health of the national parks since the publication of the Report of the National Park Policies Review Committee (the Sandford Report; HMSO, 1974).

The MacEwens set out to discover whether the national park authorities are able to preserve for the nation the magnificent conservation and scenic heritage that caused the parks to be established in the first place. They believe that it is the duty of government (both central and at the national park level) to safeguard in trust

for all citizens the many diverse scenic and natural history habitats which they claim have superior public rights to the private development rights of individual landowners. They are also very much aware that the national park landscapes are the product of care: they are not "natural" in the strict ecological sense, but a kind of semi-wild "garden" tended by generations of inhabitants to create a unique blend of cultural and

National Parks: Conservation or Cosmetics? By Ann and Malcolm MacEwen. Pp.314. Hbk ISBN 0-04-719003-5; pbk ISBN 0-04-719004-3. (George Allen and Unwin: 1982.) Hbk £15, \$35; pbk £8.50,

natural processes. The MacEwens call this characteristic "semi-wildness" and note that it has four vital elements: the opportunity for people to touch experience face to face; the repository both of natural and human history; the resource of space and openness; and a state where, until recently, man's relationship with nature has been relatively harmonious.

The MacEwens clearly regard these features as vital to the life-blood of the nation but realize that the future of the national parks cannot be separated from the future of the British countryside as a whole. Like the Edinburgh group, they are acutely aware that much of the national park land is harsh and economically inhospitable. The remote uplands are losing their working populations as agriculture is shedding labour, forestry is not attracting labour and the well-known problems of expensive and ill-provided transport lead to loss of job opportunities and services. Many of the national parks are suffering from the economic decline which is affecting remote rural Britain generally, and the local economies are so structurally weak that they cannot readily exploit the opportunities provided by tourism and the promotion of craft industries.

But the MacEwens' main criticism is levelled at the Ministry of Agriculture, Fisheries and Food (MAFF), the Common Agricultural Policy (and by implication, the Inland Revenue) for failing to recognize the social and conservation-related re-

percussions of policies favouring intensive agriculture and coniferous forestry. By means of a number of skilfully presented case studies they document how it pays landowners to exploit the public right of landscape in their own short-term private interest, aided and abetted by grants and tax incentives that positively encourage the destruction of the national park heritage. They further point out that the national park authorities are virtually powerless to stop this, lacking both the powers and the budgets to halt the decline. In addition, the MacEwens believe that the membership of national park committees should be less parochial and more committed to the original national park ideals.

But lest it be thought that the MacEwens are seeking a kind of landscape museum, one should make clear that they are anxious to extend public access to the semi-wild, and to develop the scope of environmental education and countryside interpretation so that many more people can enjoy the experiences that evidently mean so much to them. They are equally anxious to see a renaissance in rural economies favouring the retention of labour in agriculture and forestry so that key elements of the national park landscape can be retained. Indeed this is a critical point of their argument, and most relevant to any future government seeking to grapple with the problem of employment.

This means a major re-examination of MAFF policies and of the tax incentives favouring expenditures in social capital where this has both conservation and economic value. The MacEwens deplore the lost opportunity in the Wildlife and Countryside Bill debate when Lord Sandford's imaginative amendment, passed against the Government's wishes, fell in the Commons. This provision would



The price of farming? The broadleaved woodlands here in Patterdale, and elsewhere in the Lake District National Park, no longer regenerate naturally because of over-grazing.

have enabled MAFF to invest in rural enterprises to maintain employment in agriculture and related activities so as to promote conservation. The new clause is terribly weak, merely encouraging MAFF to further the cause of conservation but only within the narrow confines of their existing remit. The MacEwens are equally critical of the final shape of the Wildlife and Countryside Act with its inbuilt assumption that landowners are entitled to agricultural and forestry grants so that when such grants are denied on conservation grounds, the national park authorities will have to buy them out possibly to the tune of the profit foregone. They correctly emphasize that while these provisions are bad enough, the failure of the Act to cover the countryside outside the national parks and sites of special scientific interest could be disastrous. For here no notification is required of agricultural or forestry activity likely to be detrimental to conservation. It is hardly surprising that the agricultural community is anxious about its public image.

Like the Edinburgh group, the MacEwens conclude that the national park

authorities are toothless watchdogs, that their record has been more one of cosmetics than conservation and that the maze of bureaucratic interests with a finger in the countryside pie is emasculating coordinated action. They argue for a unification of the Nature Conservancy Council and the Countryside Commission (and possibly, eventually, the Development Commission?) on the grounds that the distinction between conservation and landscape is an unnecessary one, which may well lead to a continual weakening of the conservation cause.

There is little doubt that in the wake of the passage of the Wildlife and Country-side Act, the national park idea, already under fire because of changing economic circumstances in remote rural areas, is even more under threat. The MacEwens' arguments should be taken seriously or the nation is in danger of losing something that it will find very difficult to replace.

Timothy O'Riordan is Professor of Environmental Sciences at the University of East Anglia. He is currently preparing the rural section of the UK response to the World Conservation Strategy. ative linguists have ignored "meaning" and "understanding"? The central themes of Chomsky's work have always included the grammatical representation of thematic structure (who did what to whom with what), the referential possibilities of anaphoric elements, and the interpretation of the logical vocabulary of natural languages. There may be more to meaning than this, but surely these topics are part of "semantics" in anyone's story?

Hörmann's final point, that the study of language-use has not been advanced by recent psycholinguistic research, seems at first blush a particularly odd way of describing a period that has seen notable advances in modelling sentence parsing and production. Apparently, that area is not what Hörmann refers to as "languageuse". His fulminations are rather based upon the failure of generative linguistics and psycholinguistics "to predict events as they occur in everyday life". Hörmann's claim may be confidently conceded, although the complaint smacks of expecting Newton's laws of motion to predict when the last apple will fall from the tree in my back garden.

The bone of contention is this: common sense and Professor Hörmann know that there's a lot going on when two people talk with each other. In addition to speaking a common language (English, German, Malagassy, or whatever), the participants also bring to bear their tacit grasp of conversational maxims, and their vast knowledge of "the way the world is". Professor Hörmann notes, correctly, that theories of generative grammar do not explain everything about these "acts of meaning and understanding". But he then concludes that such theories therefore explain nothing. This hasty dismissal rather misses the point that however much extralinguistic factors may aid in understanding what someone really means, the greatest help surely comes from knowing the other person's language. It is precisely this knowledge that grammars explicate. The scope of formal grammars may, however, be very restricted. For example, Chomsky's position is that a particular level of linguistic representation - "the syntax of logical form" - is the interface between sentencegrammar and other faculties of mind implicated in the wider sense of the weasel word "semantics". Beyond the level of logical form no sharp dividing line is drawn between the "literal" meaning of a sentence and its interpretation in the light of the beliefs, knowledge and pragmatic inferences that can be brought to bear upon it. It is, of course, an empirical issue whether mental representations do indeed partition in this fashion. The pity is that Professor Hörmann obscures these topics by arguing that sentence-grammars fail to solve problems they were never intended to solve.

The prosecution of syntax

John C. Marshall

To Mean — To Understand: Problems of Psychological Semantics. By H. Hörmann. Pp.337. ISBN 3-540-10448-8. (Springer-Verlag: 1981.) DM88, \$41.95.

MUCH of Professor Hörmann's latest book is taken up with attacks upon modern (that is, Chomskyan) linguistics and the psychological experimentation that has drawn upon Chomsky's ideas. Hörmann senses that the swings and roundabouts of outrageous fashion are deserting the paradigm that has dominated the field since 1957. "It is not uncommon", he writes, "that following a period of turbulent growth, a skeptical mood builds up and doubts begin to be expressed as to the rationale of progress achieved and the extent to which the position reached coincides with the original goals".

The particular malaise that Hörmann claims to detect has three main symptoms: current linguistic models are approaching "a level of complexity at which extreme sophistication borders on folly"; these models reveal a "widening gap between linguistic theory and the realities of language"; the last 25 years of work in linguistics and psycholinguistics has "contributed very little to a clarification of the problems of language use". Hörmann's antidote, as summarized in the last paragraph of the book, is that we should study how "the hearer is driven by the intentionality of his process of living-into-theworld", how "as he passes from one level to the next, the sounds, words, and

sentences of the language become transparent" and "fade away to make room in his consciousness for the meaning meant".

I have no doubt that, sociologically, Hörmann's remarks are exactly to the point and that both his diagnosis and his remedy will strike a responsive chord in the breasts of many psychologists. Intellectually, however, the argument is more dubious. Consider first the charge of overcomplexity. No one would deny that, ceteris paribus, simple grammars should be preferred to intricate ones, but it is nature, not the linguist, that determines how elaborate the grammars of natural language are. And in any case, Hörmann seems unaware that a radical simplification of linguistic theory has taken place over the last decade; the proliferation of rules and rule-types that characterized generative syntax in the mid-1960s has been replaced by a system of very general principles whose interaction is responsible for the appearance of "phenotypic" diversity.

In order to appreciate the second complaint one must consider what "the realities of language" entail. For Hörmann,

human language is language because it is used by people for a purpose, namely, to live with other people. The purposeful use of language is embodied in acts of meaning and of understanding; in these acts the essence of language is integrated with the condition of man.

While the passage may be a trifle purple, the sentiments are surely unimpeachable. But why does Hörmann believe that gener-

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Littermates and guesstimates

Barry Cox & Michael Stoddart

The Mammalian Radiations: An Analysis of Trends in Evolution, Adaptation, and Behaviour. By John F. Eisenberg. Pp.610. US ISBN 0-226-19537-6; UK ISBN 0-485-30008-7. (Chicago University Press/Athlone: 1981.) \$45, £32.

THE theme of John Eisenberg's book is the relationship between three aspects of mammals: their anatomy and physiology, the way in which they exploit their different habitats, and their behaviour and social structure. Clearly, these aspects must be integrated in any species, but Eisenberg goes further in attempting to compare the patterns of integration in different mammalian groups, and to suggest general trends that extend from one group to another.

The first 74 pages deal with the relationship between the areas of origin of the different groups and the changing continental configurations, and survey what Eisenberg calls "the early radiations" - monotremes, marsupials, edentates and lemurs. In the next 150 pages he deals with the remaining orders of mammal in similar fashion. He is at his least authoritative in the historical and taxonomic section, for he shows the Marsupialia as already in existence in the Lower Cretaceous, while the suggestion that monotremes evolved from multituberculates that reached Australia is to found one highly dubious hypothesis upon another. After uniting the South American ungulates with such African forms as elephants, Eisenberg notes that this suggests that they had a common ancestor in those areas before the two continents separated - though the separation took place in the mid-Cretaceous, long before the differentiation of the ungulate eutherians. Furthermore, are the marsupials and edentates simply part of an "early radiation" of mammals? Since Eisenberg is attempting to deduce phyletic trees of behavioural traits, and these trees are in turn founded upon the classification and history derived from morphological studies of fossil and living forms, it is essential that he is accurate in these fundamental assumptions.

Eisenberg's method and assumptions are clearly explained on p.12.

From the initial radiations, there are today some living mammals, mostly in the tropics, that in many morphological features show relatively little modification from the original stocks... it seems legitimate to infer that certain morphologically conservative species inhabiting niches in the tropics represent forms that are in a sense adapted to a 'conservative niche'. Thus a phylogenetic reconstruction of mammalian behavioural evolution should start with generalizations developed by comparing morphologically conservative forms that occupy what might be considered the niches of the Paleocene.

He goes on to comment (p.42) that "The

age of the edentate and pholidote lineages allows them to serve as a basis for comparison with the remaining radiations of the eutherians". Finally (p.49)

If the Edentata are among the most primitive living eutherians, then the behaviour of a 'conservative' dasypodid such as *Euphractus* has great significance for comparisons with other morphologically conservative mammals. Unfortunately we have virtually no field data . . . We must therefore rely heavily on captive studies.

The gaps in this chain of reasoning are obvious. To what extent is any niche today equivalent to a niche in the Palaeocene? Are armadillos really morphologically conservative? If they are, at least in those characters that condition their ecological niche, to what extent does this allow us to deduce that they are also conservative in their reproductive and behavioural strategies? How much meaning is there in the concept of a "phylogenetic reconstruction of mammalian behavioural evolution"? These are important reservations, that must be raised now because they counsel caution in reading the rest of the book.

In Chapters 17-23, Eisenberg discusses what he terms "macrophysiology and adaptation" — an integration of dietary specializations and ways of life. This is the classical stuff of mammalian adaptive radiation, and his approach is useful and thought-provoking. For example, why are fossorial niches incompletely occupied in the tropics, and why are more "macroniches" filled in North America than in Europe or North Asia?

In the final 100 pages of text, Eisenberg develops the theme that there was originally a common basic mammalian behavioural repertoire, which has been adapted through natural selection in the

various diversifying lineages. He first attempts to identify this repertoire by comparing various patterns of behaviour in terrestrial mammals; this chapter is brief and rather unsatisfactory. However, in a detailed and most valuable analysis of social organization, Eisenberg spiritedly tries to correlate such behavioural systems as mating, rearing, foraging and refuging. He then extends his net to show relationships between demographic, ecological and sociological variables in a wide range of species. The use of arbitrary numerical values for qualitative characteristics (e.g. "reproductive rate" is scored from 1 to 7) is understandable but can be misleading. Many species vary their reproductive output and adopt a slightly different social organization at different altitudes or latitudes, so the reader must beware of these oversimplifications. However, the author seems more at home in this section than in the earlier parts of the book, and his extrapolations should fuel many a coffee-room debate.

There is an excellent and wide-ranging bibliography of over 1,700 references, and Eisenberg has had the happy idea of entering many of these in group versus subject tables that show, for example, references on rhinoceros behaviour or tapir ecology. Appendices, classifications of extant mammals and an index complete the book.

For research workers and postgraduate students this is certainly a stimulating book, full of data, correlations, extrapolations and provocative guesses. It is therefore dangerous material for the unwary or inexperienced, who may be unaware of the fundamental assumptions that Eisenberg's thesis is founded upon, and may be slow to appreciate the boundaries between correlation, extrapolation and guesswork.

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Life of the long-haired stars

David W. Hughes

Introduction to Comets. By John C. Brandt and Robert D. Chapman. Pp.246. ISBN 0-521-23906-0. (Cambridge University Press: 1982.) £21, \$45.

COMETS are heavenly bodies of considerable beauty, volatile character and deep physical and chemical significance. Their contents are fresh, thermally unaltered and undifferentiated, and the large outpourings of gas and dust that occur when the comet is close to the Sun often produce a coma and tail of truly astronomical proportion. Comets are also newsworthy. The past decade has seen the

blossoming of ultraviolet and infrared comet research; the next will see the launching of the first spacecraft designed to pass through the head of a comet and to gather *in situ* data.

Monographs on comets are scarce and there have been only three major ones since the turn of the century: G.F. Chambers's The Story of the Comets (Oxford University Press, 1910); R.A. Lyttleton's The Comets and their Origin (Cambridge University Press, 1953); and N.B. Richter's The Nature of Comets (Methuen, 1964—an enlarged translation of his 1954 book, Statistik und Physik der Kometen). We

now have Brandt and Chapman's Introduction to Comets. Let me say straight away that a very good introduction it is too; a worthy and timely fourth addition to the list. The authors have conveyed the excitement of modern comet work, the pace of the data flow and the originality of many of the new theoretical approaches. They are excellent recruiting agents for the subject.

The book starts by placing comets in the historical perspective and reviews many of our modern ideas about comets by discussing how scientists first approached, for



Halley's head, photographed on May 8, 1910, from the Hale Observatory.

example, cometary spectroscopy and the interactions of comets with the solar wind. The second section deals in depth with the dynamics and structure of comets and looks at the models that have been proposed for the chemical reactions and dust emission processes. The origins of comets and their decay into meteor streams are also considered. The third section reviews the enormous advances that have come from the investigation of some of the recent bright comets, such as Kohoutek, Kobayashi-Berger-Milon and West. It also discusses the possible spacecraft missions to comets. The last section briefly touches on comet germs and impacts, and the place of comets in literature and art.

The book is extremely well illustrated and delves into the nature of comets with sufficient detail to satisfy the average undergraduate and postgraduate reader. Those seeking more, are efficiently led to an impressive list of publications in the "suggested reading" section.

I think the authors have used their 246 pages most effectively. For my taste I would have put in more than three pages on origin mechanisms, and would have laid more emphasis on orbital evolution, decay process and size variation. Also the section on cometary missions seems to have been written long before the publication date, in the rosy times when NASA were still actively planning cometary missions. The people who *are* going to Halley in 1986 don't get a mention.

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Progress and prospects in solar physics

J. C. Brown

Solar Active Regions: A Monograph from Skylab Solar Workshop III. Edited by Frank Q. Orrall. Pp.350. ISBN 0-87081-085-5. (Colorado Associated University Press: 1981.) \$17.50.

IDEALLY the trilogy of Skylab Solar Workshop proceedings would have progressed from the tranquillity of Coronal Holes through Active Regions to achieve its nexus in Solar Flares, thereby exhibiting the physical progression of solar phenomena. In reality even the comparative opulence of workshop funds, albeit a tiny fraction of mission costs, did not permit this degree of coordination. Nevertheless the substantial overlap of participant lists means that the three workshop volumes together contain useful interdisciplinary seeds for the solar activity student to cultivate. Benefiting perhaps from previous experience, this third volume is the most readable.

The editor opens with a thorough overview of workshop results from each of the five teams who contribute about two detailed chapters each. A recurring theme throughout is theoretical and observational modelling of static loops of which the corona is now held mainly to consist. It appears to be generally accepted that spectral analysis can shed only dim light on details of loop heating mechanisms, though scaling laws permit comparison of the magnitude of heating in relation to loops of different lengths and pressures. Aside from these static analyses, the work includes a lengthy theoretical discussion of gas flows and thermal instability in loops, complemented by two chapters on observations of dynamic phenomena at chromospheric/coronal levels and near the photosphere, the latter invoking data from OSO-8 as well as from Skylab

For the non-specialist the chapters of greatest interest will be those on theoretical coronal heating mechanisms and on spectral diagnostics, both of which are lucidly presented. The former contains much on energy release in plasmas which will interest plasma astrophysicists in general, while the latter concisely reviews the principles of spectrum line diagnostics for plasma temperatures, densities and differential emission measures. A frank assessment of progress made and of prospects for future work on active regions completes the monograph.

As usual this workshop raised more questions than it resolved, reiterating the stark solar message for cosmic astronomers of just how difficult astrophysical problems become when the object of study is close enough for detailed scrutiny. The Skylab Workshop series presented a unique opportunity for thrashing out both theoretical and observational progress in

important specialized problems. It is to be hoped that the sorry current state of NASA funding does not preclude such in-depth attacks on data from more recent missions such as Solar Maximum.

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Sex differentiated

W.K. Whitten

Mechanisms of Sex Differentiation in Animals and Man. Edited by C.R. Austin and R.G. Edwards. Pp.603. ISBN 0-12-068540-X. (Academic: 1981.) £37, \$89.

In 1970, Harris and Edwards edited the Royal Society's discussion Sex Determination, and in 1972 a chapter by Short entitled Sex Determination and Differentiation appeared in Reproduction in Mammals, edited by Austin and Short, and published by Cambridge University Press. Now Austin and Edwards restrict their title to Sex Differentiation and argue that determination is the initial step in homotypic differentiation. But this is not accepted by some of their contributors, so the editors conclude that the argument is unresolved — a healthy attitude, emphasizing the need to standardize terminology.

This volume of 12 chapters written by specialists is intended as a text for advanced students and researchers. Lyon, Cattanach and Charlton give superb descriptions of testicular feminization, XY female wood lemmings, and sex-reversed and hypogonodal mice. The study of these conditions and the H-Y antigen, described by Wachtel and Koo, have contributed greatly to the recent explosion in our understanding of sex differentiation. There are excellent chapters on embryonic development and function, postnatal growth of gonads and sex characters from the clinical point of view, and the many defects in determination and differentiation in human beings. The structure of the gonad, however, is followed only as far as initiation of meiosis. I assume that this is because folliculogenesis and gametogenesis are covered in other recent books such as Peters and McNatty's The Ovary published by the University of California Press in 1980. There is a review of X-chromosome inactivation, but unfortunately no contribution by Ohno who anticipated many of the recent advances.

Benirschke adequately reviews freemartins and hermaphrodites of large animals, but his treatment of mosaics and chimaeras of mice is poor. Apparently he has not read Russell's Oak Ridge Symposium volume, Genetic Mosaics and Chimeras in Mammals (Plenum, 1980), nor the more recent work with fetal mouse hermaphrodites. These topics really deserved a chapter by Beamer whose BALB/CBea mice generate mosaic hermaphrodites by nondisjunction, or Eicher who has transferred the Y-chromosome of Mus poschiavinus to C57BL/6J-YPOS. This chromosome carries a variant of the testis-determination locus which interacts abnormally with autosomal or X-linked loci preventing normal testis differentiation even in the presence of H-Y antigen. Also there are excellent controls from consomic strains for these interesting animals.

The words "mechanisms" and "animals" in the title are misleading because some chapters, such as Stevens's account of his exciting spontaneous and induced teratomas, are purely descriptive, and except for a few pages of introduction and an excellent contribution on environmental and non-genetic mechanisms the book is restricted to mammals.

Reviewing this book has been difficult because many of the references cited, including books, are recent and not freely available in libraries with restricted budgets. There is no author index and the inadequate subject index appears to have been compiled from the table of contents. An editorial policy of giving the specific name when a species is first mentioned would have ensured entries for "man" and "mouse", at least in the species index, and eliminated some redundant ones. Surely textbooks warrant the services of professional indexers?

The editors consider the possibility of voluntary control of sex determination and doubt the "dire prognostications" that acquisition of this knowledge would upset the "fabric of society". Instead, they predict smaller, planned families and elimination of sex-linked disease, but conclude that no foolproof method exists at present. Already blastocysts can be sexed before implantation and fetuses of the unwanted sex aborted. Ironically, the early data from test-tube babies gave an excess of females (P<0.03) which might have been interpreted to mean that a procedure favouring girls had been stumbled upon. But I believe that biologists generally should require a probability of less than 0.01 before assigning significance, and this conviction has been supported by more recent results, particularly from Melbourne.

In spite of these criticisms I consider this a valuable and major work, updating Crew's little gem Sex Determination, published by Methuen in 1965, and fit to stand alongside White's Animal Cytology and Evolution (Rand McNally, 1973).

News of conception, pure and applied

C.R. Austin

Fertilization and Embryonic Development in Vitro. Edited by Luigi Mastroianni and John D. Biggers. Pp.371. ISBN 0-306-40783-3. (Plenum: 1981.) \$45, £28.35. In Vitro Fertilization and Embryo Transfer. Edited by E.S.E. Hafez and K. Semm. Pp.393. UK ISBN 0-85200-438-9; US ISBN 0-8451-3005-6. (MTP Press/Alan R. Liss: 1982.) £29.95, \$58.

THE recent upsurge of attention paid in the public media to the growing number of normal childbirths following the fertilization of human eggs in vitro, and the culture and reinsertion of the resulting embryos into their "mothers", must serve as welcome publicity for these two books, both of which provide in their own way the background to those newsworthy events. The publicity is a late development, for the vigour of scientific research in this field has been building up for the past 25–30 years, and the intellectual springs behind this drive are based on biologists' ambitions that go back a century or more.

Some of the history is discussed in the Prologue to the book edited by Mastroianni and Biggers, though this account is concerned primarily with milestones along the trail of the recovery and culture of mammalian embryos. From early times, investigators have been fascinated by the prospect that conditions might be achieved in the laboratory permitting the union of mammalian gametes and the ensuing development of the embryos in such a way that these events could be held under direct observation in vitro. Also early in the story, the remarkable resilience of mammalian embryos to experimental manipulation attracted devoted study, and the first successes with the transfer of embryos from one animal to another were achieved before the turn of the century. With increasing sophistication the focus has sharpened in the modern era so that investigators have become concerned more and more with fundamental details.

The two books under review have, not surprisingly, much in common. They are of about the same vintage, with that edited by Hafez and Semm marginally the older as it is based on papers presented at a world conference in 1980. The other is a "planned" book of 15 chapters (21 authors, all in American laboratories), while Hafez and Semm is comprised of 37 papers (71 authors, of whom 51 work in countries other than the USA, chiefly West Germany, France and Japan). The common ground of subject matter includes the recovery and preparation of gametes, in vitro fertilization, the culture and evaluation of embryos, and embryo transfer; differences arise from the manner in which the subjects are handled and in the identity of peripheral topics. Broadly

speaking, Mastroianni and Biggers is biased towards basic problems and Hafez and Semm towards the applied.

The former volume is distinguished by a monumental chapter by R. Yanagimachi on mechanisms of fertilization in mammals; this, together with three others (by G. Oliphant and L. A. Eng, B. D. Bavister, and D. P. Wolf), tells the reader almost all there is need to know about sperm maturation and capacitation, the acrosome reaction, penetration of egg investments, sperm-egg pronuclear life and the blocks to polyspermy - all this in laboratory animals. In vitro techniques are stressed. In addition, this book offers two chapters on gamete interactions and egg activation in the sea urchin (a worthy comparative background, though inessential in the present context), two chapters on chromosomal anomalies affecting embryonic development, two more on the rather esoteric topic of water and electrolyte balances in blastocysts, and finally a well planned and informative chapter on embryo transfer in cattle.

In Hafez and Semm, by contrast, no less than 14 papers describe the application of methods for in vitro fertilization, and four deal with embryo transfer. About half the papers in this book accord at least a mention to the human subject, for whom the timing of ovulation, oocyte recovery and maturation in vitro, fertilization in vitro and embryo evaluation are treated in varied but generally satisfactory detail, and the ethics of embryo transfer are briefly discussed. Curiously, none of the research groups that have reported full success with human material - well established pregnancies and normal births - are represented here. Other papers are concerned with non-human primates and farm animals. The book also fortunately provides us with Pierre Soupart's report, bringing us up to date with his work on initiating development by the singular device of fusing two oocytes; this may have been his last paper. In addition, there is a report on gonadotrophin production by baboon embryos in vitro from V.Z. Pope et al., and descriptions of two recently developed rapid assay methods for LH by M. Taymor et al. and by R. Frydman et

Both of these books are mines of information and "musts" for all gametologists; if only one is to be purchased the choice must depend on the predilection of the reader — whether for the basic or the applied, for the transatlantic or the international, or simply for the more or less expensive.

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In search of the sublime

S. M. Walters

The Garden of Eden: The Botanic Garden and the Re-creation of Paradise. By John Prest. Pp.122. ISBN 0-300-02726-5. (Yale University Press:1982.) \$25, £12.50.

AMONGST the ever-growing literature on the history of gardens, relatively little explicitly treats of botanic gardens and their place in the general development of horticultural ideas and practices. It is therefore of some interest to note the subtitle of John Prest's book, and to see how far the text which accompanies the lavish illustrations lives up to the promise. The thesis of the book is interesting, even novel: he traces the idea (or more strictly ideas) of Paradise in Christian thought and imagery as reflected in the design of gardens through the centuries, and places the sixteenth and seventeenth century botanic gardens firmly in this tradition. Whilst it is easy to agree that Christian images and influences were powerful in gardening history, the thesis does not wholly convince, for it underestimates the scientific and instructional aspects of the work of botanic gardens.

As befits an Oxford historian, the author has chosen the oldest botanic garden in England, that in his university, to illustrate the shape and scope of early botanic gardens, but it is surely too parochial altogether to ignore Kew except for a single, oblique reference. The final chapter, which discusses the breakdown of isolation of the hortus conclusus in the new climate of interest in nature and the natural world, is surely the place where Kew Gardens cries out for treatment. The interplay of botanic gardens with the great private gardens of the nobility is indeed a fascinating theme, but it is curiously and disappointingly undeveloped in this book.

The relationship between the plants and the animals in the Garden of Eden — one of the splendid illustrations in colour (also used on the dust-jacket) depicts this scene painted by Jan Brueghel - is touched on throughout the book, with the strong implication that it was only the sheer impractibility of including animals that prevented botanic gardens from being also zoos. This is surely a serious distortion of the history of botanic gardens, which arose largely to satisfy the needs of medical teaching in the universities. Although it is true that botanic gardens later took on the general function of displaying as wide a range of plants as possible, there is no evidence that they were ever seriously troubled by the vision that they might also display a correspondingly wide range of

A second edition of Sarnat and Netsky's Evolution of the Nervous System has been published by Oxford University Press, price £9.95. The first edition was reviewed in Nature 252, 428; 1974.

representatives of the animal kingdom. Why should they, when systematic zoology was not taught in the universities, either to medical students or to anyone else?

It is perhaps inevitable that a book written by a historian on botanic gardens should say very little about the plants themselves, although they are the *raison d'être* of the institutions which grow them; when we find, however, that such few Latin scientific names as are used are mostly given incorrectly, with no capital letter for the generic name (as on pp.61 and 83), our confidence in the author's knowledge of this part of his subject is seriously undermined.

Undoubtedly, the author has read both widely and deeply in and around his subject, as the many footnotes, sensibly collected at the end together with the excellent bibliography, bear witness. As a source for further exploration of interesting themes, the book is quite exceptionally useful. The quality of paper, printing and reproduction of illustrations is very high, and the price, by 1982 standards, quite modest. A minor irritation is that many of the pages are unnumbered, because of the placing of illustration captions: this could surely have been avoided, without detriment to the very attractive design.

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Chemical soul

A.G. Lee

Liposomes: From Physical Structure to Therapeutic Applications. Edited by C.G. Knight. Pp.497. ISBN 0-444-80320-3. (Elsevier/North-Holland Biomedical: 1981.) \$110.75, Dfl. 260.

THE behaviour of suspensions of phospholipids in water (liposomes) has been under study for many years now, originally as a model for the lipid phase of biological membranes but more recently to investigate the possible therapeutic applications of liposomes in the delivery of drugs, enzymes and so on.

It was apparent by the late 1960s that the fact that lipids formed sealed vesicles when dispersed in water meant that they might be used to trap and protect unstable compounds from a harmful environment and so, for example, might enable the oral administration of compounds that would otherwise be inactivated in the gastro-intestinal tract. One possible advantage of

liposomes for such a role is that since they are composed of "natural" compound, they might be expected to have little biological activity on their own. It has not turned out to be quite so simple. The problems, and attempts to overcome them, comprise the second part of this book.

Unfortunately, liposomes have antigenic properties, described in a chapter by Scherphof and co-workers. Patel and Ryman discuss the fate of liposomes when administered to animals, and Finkelstein and Weissmann describe attempts to target liposomes to specific tissues. An obvious problem raised in several chapters is that for liposomes to act as efficient drug delivery systems, drugs must not readily leak out of them. As discussed by Knight, this can in part be overcome by designing hydrophobic pro-drugs which are more likely to remain associated with the liposomes. In part also, it can be overcome by choosing components for the liposomes which will minimize their "fluidity" Nevertheless, the conclusion reached by Fildes is that the application of liposomes in the pharmaceutical industry is a long way off, because of a wide range of technical problems. At their most basic, liposomes would not be expected to last for very long in the average bathroom cabinet.

The overall impression of the second half of the book is then, as far as therapeutic applications are concerned, that liposomes are an answer awaiting a problem. With the first half of the book, we are on much more solid ground. This sets out to describe the physical properties of liposomes, with the aim of providing the information necessary for the proper design of liposomes for therapeutic applications. I imagine, however, that the book will be read mostly by those with an interest in biological membranes. It does provide an excellent, non-technical summary of our knowledge of the lipid bilayer, although the lack of discussion of the effects of proteins perhaps limits its general interest.

As is proper, the first chapter is a general introduction by Alex Bangham, who has done so much to generate the current interest in liposomes. A chapter by Eibl describes the synthetic routes to a wide range of phospholipids that he has developed based on reactions of phosphatidic acid dichlorides. We then have chapters on freeze-fracture studies, differential scanning calorimetry, X-ray, NMR and ESR. The latter chapter, as is appropriate for ESR, has an aura of mystery about it — the journal volume numbers given as $c \ll 1$,

Finally, readers will have to decide for themselves whether or not Thudicum's description of lipids as "the centre, life and chemical soul of all bioplasm" (quoted by Bangham) is a little over the top.

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nature

29 April 1982

What place for social sciences?

Lord Rothschild's enquiry into the British Social Science Research Council seems to be complete but the report has not yet been written. Here is what he should conclude:

In many places social science is regarded as integral with science itself, under some such name as Wissenschaft. In Britain, by contrast, the social sciences have been regarded as studies apart and as studies that are inherently poor relations of the natural sciences. There has been a research council to support academic research in the social sciences for only the past two decades. During that period the Social Science Research Council has earned a mixed reputation. Like its counterparts elsewhere, it has found that many of the projects it has been supporting can easily be made fun of. It has also — and openly — proclaimed that part of its task has been to improve the quality of academic research in the social sciences. (In the process, inevitably, some of its functionaries have been persuaded to laughable pedantry.) Then, with the expectation that research should be useful, the research council had been asked insistently the old utilitarian question, "what use is it?". Nobody knows just which of these views persuaded Sir Keith Joseph, the Secretary of State for Education and Science, to let it be known that he is sceptical of the council and most of its works and, soon after his own appointment last September, to saddle Lord Rothschild with the task of pronouncing on the council's future. Perhaps he was simply counting on the likelihood that the man who caused ructions among natural scientists with his recommendations in 1971 for the reorganization of the other research councils would be equally subversive in the social sciences.

What, however, should he say? The first need is to recognize that the abolition of the Social Science Research Council would be intolerable. Whatever its hesitancy in the past two decades and its ways of working, the council has not merely established a number of important and productive academic groups in British universities, but it has substantially achieved that part of its programme intended to improve the quality of research in the social sciences. Moreover, especially at a time when the universities as such are less able than ever before to support research, the abolition of such a serious fund as the research council has become would create much more damage than the universities could sustain.

So, should the Social Science Research Council be reorganized in some way, and if so, how? In the past three years the council has been doing everything it could to make its procedures more understandable to those whose work it supports and at the same time to make its intentions more widely appreciated by the government and in the country at large. In the process the council has angered many of those who had grown used to the old procedures. Thus the council now finds that its critics are in two camps — those who preferred the past, and those who are unconvinced that its present procedures will deliver what people expect of the social sciences.

Nobody should be dismayed that this turn of events has come about. Who asks that a newly created organization such as the council should be able, within a couple of decades, to find a sure

US contributors please note

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way of supporting research in the social sciences? The problems, acknowledged by everyone concerned at the outset, are too serious for that. And in any case, the successes in the council's portfolio of previously supported projects are quite sufficient to outweigh the hasty criticisms of those who point to projects that have failed. If some reorganization of the social sciences is needed, and it may be, attention should be directed to the present mismatch between what the council does in the support of academic research and the uses which government departments make of academic skill and research in the social sciences.

One of the most valuable parts of Lord Rothschild's declaration on the Social Science Research Council will undoubtedly be the calculation he will have made of the amount of research in universities commissioned directly by government departments and that supported by the research council. Paradoxically, the circumstances are the inverse of those that stimulated Lord Rothschild's recommendation of 1971 for the natural sciences. There, a decade ago, research councils such as those responsible for agriculture and medicine were free to decide for themselves what research strategy should be pursued, while the ministries most directly concerned were able only in passing to offer advice. The recommendation, immediately accepted by the Heath government, that in future (as now) the ministries should commission research in important areas from the research councils was sensible. The fact that the "customer-contractor" principle has not worked entirely satisfactorily is no fault of the principle, but of those who have tried to apply it. In the social sciences, on the other hand, the volume of research commissioned directly by government departments is far greater than that which the Social Science Research Council is able to support. Moreover, on the face of things, the projects commissioned directly by government ministries are put out to academic research groups with comparatively little advice from the Social Science Research Council, the body best equipped to tender an informed opinion. So is there not in the future organization of the Social Science Research Council, a need that the whole volume of public support for research in the social sciences should be channelled through some common agency?

The question remains of what should be the relationship between the social and the natural sciences. Academics, notoriously hide-bound, have over decades argued the toss about the place that social science should occupy in academic life. Economics is accepted, now, as an inevitable part of the spectrum of what goes on in universities. More esoteric subjects anthropology on the one hand, sociology on the other hand - are more easily scorned, but wrongly. Physicists may hold that the data on which people in these disciplines base their arguments, and the arguments themselves, are fuzzy by the standards that apply in, say, the analysis of data collected in some objective measurement of the real world. But was there not a time when even physics was a fuzzy field of speculation? Can it be right that the natural sciences should continue to laugh at the difficulties with which the social sciences must contend? For what the field needs is what the Social Science Research Council has been saying for the past twenty years is an essential part of its own brief — the general improvement of methods of teaching as well as of research that will, in due course, make social science accessible to a wider circle, and thus more useful.

"Freeze" frenzy hits US

Does the public demand for a nuclear weapons "freeze" reflect a basic shift of opinion?

Enthusiasm for a freeze on the production of nuclear weapons is sweeping the United States. Rallies and teach-ins abound, with participants waving pictures of Hiroshima victims and charts of death from "limited" bomb blasts. Students and faculty on many campuses are involved. So are physicians. Even in the southern states, which was fairly unmoved by the Vietnam protests, freeze frenzy is taking hold.

The movement has been accompanied by pronouncements from prominent people calling on the Reagan Administration to stop its weapons build-up, and blaming the arms control and national security community for giving the public gobbledygook and no results for its 20 years of effort. Historian Barbara Tuchman, whose ability to understand the intricacies of history has won her two Pullitzer prizes, threw up her hands in pages of the New York Times recently and confessed that "the subject of nuclear arms control . . . is virtually incomprehensible to the layman". She showed how arms control has failed since the beginning of the century. Meanwhile, Yale psychiatrist Robert Jay Lifton has blamed the intellectual community for not doing enough. "This central issue of our times has been fundamentally ignored in the universities", he said at a recent conference jointly sponsored by two education groups and Hobart and William Smith College. The failure of leaders and the security community was bemusedly outlined in public recently by a former participant, Roger Molander who after years working on nuclear arms control in the White House quoted a presidential science adviser as saying, of the leaders' behaviour "where are the grownups?" Molander quit and founded "Ground Zero", a grass-roots organization that has been remarkably successful in sparking rallies around the land.

Are we witnessing a fundamental shift in US opinion? Europe is watching it closely, particularly the European peace movement, which is hoping that it will dissuade the Administration from its hawkishness, and perhaps tip the balance against NATO plans to install new missiles in Europe.

But the shift may not be fundamental. It is caused by two things, both of which can change quite soon. First, the President and Mr Caspar Weinberger, Secretary of Defense, and other officials, have been saying things in public that previous Administrations were wise enough to avoid. They have talked openly, and casually about the likelihood of nuclear war, the "fact" of Soviet military superiority, the need for a gargantuan, apparently ceaseless weapons build-up, and they have appeared patently insincere about arms control. By escalating public discourse, clearly they are upsetting people.

The second cause of the freeze frenzy is the volatility of public opinion itself, which is now swinging back from its hawkish mood of recent years. The last time a serious arms control plan was presented to the public, SALT II in 1979 and 1980, they were clearly bored. Then the humiliation in Iran, and Soviet invasion of Afghanistan, sparked a conservative trend which eventually brought Reagan to office. If the public is asking why, today, there is not sufficient arms control, it has itself, in part, to blame.

European peace movements should realize how different the American freeze movement is. The US demonstrators seem not too concerned about the proposed emplacement of Pershing and cruise missiles in Europe — the 1979 NATO decision which Europe's peace movement wants reversed. To the Americans, those Pershing and cruise missiles are small fry compared with Reagan's plans for the MX missile and an apparently ceaseless parade of giant aircraft carriers, submarines, nuclear-armed B-1 and Stealth bombers, and the like which the American movement seeks to freeze. Nor is the neutron bomb a major US issue. The two movements have not fused, although some major gaffe by the Reagan Administration could yet cause it to happen.

And Americans should question whether their intellectuals are

to blame - whether, as Lifton grandly said, "a generation of scholarship has been lost" — whatever that means. To blame the arms control and national security intellectuals for the military build-up in the world is like blaming social scientists for the persistence or racism, poverty, and violence. True, one of the vanities of intellectuals is that they think that by studying a subject, they can control it. But not even scientists can do that. William Perry, former Under-Secretary for Defense Research and Engineering, and one of the key defence intellectuals who tried to "sell" SALT II for the Carter Administration, says there is "some merit" in the view that US defence intellectuals have failed the country. But it was the public's fault too, he notes "very little else at the time (besides SALT II) was guaranteed to put the public to sleep". Perry welcomes the freeze movement although he disagrees with some of its aims. "As a leader in an Administration I would rather work with an aroused public, and try to channel that interest, than have to try to convince an apathetic public.'

It would be unwise for the Reagan Administration to view the freeze frenzy as a suspect foreign import, festering on US university campuses, and accuse its adherents of lack of patriotism. While the movement has set itself against the Administration's arms build-up, it would clearly be delighted by any presidential move to talk seriously about arms control with the Soviet Union. For the first time in years arms control is gaining a public constituency in the United States. It is too bad that SALT II is not before the Senate, so that the United States, Europe, and the Soviet Union, could benefit from it.

Hasty abolition

Disappearance of the Schools Council was predicted, but may have been unwise.

The British Secretary of State for Education and Science, Sir Keith Joseph, is plainly an abolitionist by temperament (see preceding page). Hankerings to get rid of the Social Science Research Council are one thing. Last week, however, he abolished the Schools Council, an organization set up in Britain in the 1960s in the hope of overcoming some of the obstacles to national planning in British schools education. For in Britain, as elsewhere, the central government has no direct authority over teachers in local schools, who are employed by and responsible to their local education authorities. The result is that the curriculum can vary enormously from one place to another; school leaving examinations can differ in stringency from one examinations board to another; and in principle there is no reason why some local authorities should not give their schools entirely eccentric marching orders. The problem of bridging this gap has daunted many governments and yet is politically insoluble — the local authorities in Britain would not sacrifice such independence that they retain in this small area of their operations.

The Schools Council was designed principally as a talking shop, a place where central government, local government and independent people could be represented and in which, tacitly at least, local authorities and the central government would come to some understanding on education policy. What has gone wrong is that the Schools Council's procedures have become cumbersome and the council itself has been politicized by the conflicting interests it represents, often those of a teachers' labour union. At the same time, the original plan to bring about some coherence in British schools education by advising novel and more stimulating curricula has been defeated by the shortage of funds and, to some extent, by the apathy of the schools. The Schools Council has in the past few years been a sitting duck, ripe for abolition. Sir Keith Joseph has done this deed and now plans to replace the Schools Council by two advisory bodies, one for the curriculum and one for examinations. The snag is that these advisory bodies will not, by their constitution, bring central and local government together. When he is more experienced in his present post, Sir Keith Joseph will regret this.

US biomedical research threatened

New director at NIH faces discontent

Washington

The primary sponsor of US biomedical research, the National Institutes of Health (NIH), is at last, after a ten-month hiatus, getting a new director. He is Dr James B. Wyngaarden, whom the Administration and the biomedical community agree is a good choice. Wyngaarden had his Senate confirmation hearing last week and is expected to be confirmed and sworn in within the next few weeks. But he will be inheriting a storm.

When Wyngaarden moves in to NIH's blossoming campus on the fringes of Washington, he will have to face the anger of deans and administrative officers of the 2.500 medical schools and universities in the United States who receive NIH funds and who are furious with NIH for proposing to cut their indirect cost allowance by 10 per cent in 1983. Although the immediate sum involved is a trivial \$70 million in NIH's proposed \$3,700 million budget, the administrators fear that the cut portends a fundamental shift in the structure of the US university economy. Whether it does or not depends on Congress, which may yet restore the money.

Wyngaarden, 57 and a Democrat, is Hanes Professor of Medicine and chairman of the department of medicine at Duke University School of Medicine in North Carolina. Among other Washington assignments, he has served on the President's Science Advisory Committee. His nomination was backed by NIH's many supporters on Capitol Hill, and by Richard S. Schweiker, the chief of NIH's parent Department of Health and Human Services, who is himself a former congressional backer of NIH. Although some of the Reagan Administration's key science appointees have included unknowns or outsiders to the basic science community, Wyngaarden is neither.

Wyngaarden's first job will be to find directors for the five NIH institutes which are without permanent directors, some since January 1981. Another key post, that of deputy director of NIH for science, is also vacant. The previous director, Donald Frederickson, drew up lists of candidates last July, and Wyngaarden will have to see how many candidates are still interested in the jobs. In federal research agencies, when a permanent chief is lacking, normal business continues. What suffers is the organization's internal needs and long-term planning.

Wyngaarden inherits a dispute over indirect cost allowances that is dividing university administrators and faculty across the country. The bulk of NIH funds for research project grants and research centres is awarded in two parts. The first part is the direct cost of the research salaries, equipment, travel, publications, and the like. The second part covers indirect costs, a percentage of the direct costs that the government agrees to pay to the researcher's home institution to keep him housed. Indirect costs include such things as central fuel and electricity bills, cleaning, library and central animal facilities.

Previously NIH reimbursed the institutions a fixed 15 per cent of a researcher's direct costs, no matter what they were. However, this was deemed arbitrary and NIH allowed the percentage it would repay to grow, frequently reassessing it to take account of rising utility costs and inflation. For 1983, NIH estimates that indirect cost payments on research grants and centres will average 30 per cent of the direct costs. Other federal agencies that fund research follow the same process but with percentages that vary among agencies and universities. (The

resulting patchwork of repayments would be unbearable for university administrators were the funds not essential for their hard-pressed institutions.)

Thus, while the growth in research budgets has tended to slow in recent years, indirect costs have kept on rising. A Columbia professor estimated recently that fifteen years ago his research supported by NIH enabled Columbia to collect \$2,000 in indirect cost repayments. During that time, even though his research effort has only doubled, Columbia has been able to collect \$40,000 in indirect cost reimbursements on it.

However, the array of administrators and their representative societies, such as the Association of American Medical Colleges and the Association of American Universities who oppose the NIH plans to reduce its contribution to administrative costs, are not merely concerned about paying next year's heating bills. They claim that NIH is shifting the whole debate about what the institutes are for and how they should maintain US world leadership in biomedical research.

During his six years as NIH director, Frederickson argued that researchers suffered from uncertainty as to what would

Franco-Soviet space flight plea

Three pressure groups of French scientists and intellectuals, "Comité des Physiciens Français", "Comité Sakharov" and "Solidarité au Spacionaute", have called for France to withdraw from the Franco-Soviet manned spaceflight planned for June.

Following the procedure of the manned Interkosmos flights, which included representatives of all Comecon block countries, two French candidates for space — Jean-Loup Chretien and Patrick Baudry, are now training in the Soviet Union. One of these will be selected to be launched with a Soviet partner to rendezvous with and work aboard the Salyut-7 space station put into orbit last week.

The pressure groups base their demand on the recent increase of human rights violations in the Soviet bloc — the invasion of Afghanistan, the banishment of Sakharov, martial law in Poland. For a relatively minor scientific gain, they say, France would be reduced to a "supernumerary" in a Soviet public-relations spectacular. The propaganda value to the Soviet Union of a joint flight involving, for the first time, a guest from outside the Socialist bloc, would be immense.

For all three groups agree that at this stage France stands to gain little from the proposed manned flight. Franco-Soviet cooperation in space goes back to the visit of President de Gaulle to Baikonur in 1966,

and over the years has included a laser ranging experiment on the lunokhod Moon-rover, gamma ray spectrometers carried aboard Venus probes, and the launching by the Soviet Union of the French Aureole geophysical satellites. French participation is also planned in the Soviet mission to Venus and Halley's comet in 1985.



The proposed French participation in the Salyut mission would cover three main fields, astrophysics, materials sciences and biology. Comité de Physiciens Français, in particular, contends that the first two programmes could be carried out equally well aboard an unmanned automated spacecraft. As for the biology programme, this will simply allow the "well-known" physiological stresses of space to be applied for the first time to a French citizen.

Vera Rich

happen to the research budget from one year to the next. This discouraged bold thinking and long-range research plans, he said.

According to Frederickson, NIH should fund 5,000 new "noncompeting" research project grants, in addition to keeping previous years' commitments to projects lasting two and three years. In this way, researchers would know there would be 5.000 research project grants available for the best proposals. As a corollary, Frederickson proposed that training grants should be stabilized, with the budget allowing for 10,000 of these, each year. The numbers were arbitrary and widely known to be a budget ploy, but they had the salutary effect of keeping everyone's attention on a few key concepts for the health of research: stability, availability of money for new projects, encouragement to young researchers.

Sources close to the NIH budget process say, however, that last December, when NIH received a 1983 budget figure, it calculated that it would allow funding for only 3,500 new research project grants, embarrassingly far below the 5,000 goal publicized by Frederickson, and well below the 4,741 new project grants funded in 1982. Schweiker therefore told NIH to change the budget, keeping the same total, so that the number of new project grants would be above 4,000. As a result, NIH proposed taking the cut in the administrative side, in direct costs, to avoid an enormous fight with scientists claiming that NIH was abandoning its commitment to innovative research.

So now the universities and research institutions are asking why they should be singled out for cuts — and what Pandora's box has been opened for future years.

According to John F. Sherman, former deputy director of NIH and now vice-president of the Association of American Medical Colleges, which protested to Schweiker in March, it is likely that the 10 per cent cut proposed for 1983 will set a precedent for unpredictable cuts in NIH's indirect cost allowances in future years, and perhaps lead to a lowering of indirect cost allowances by other federal agencies.

"If there is anything to divide faculty from academic administrators, this is it", says Sherman. "The institutions' representatives are frantic. They see no solution except for Congress to add the money. Whereas the faculty are more willing to see indirect cost reimbursements cut in order to maintain a higher number of competing research awards."

If NIH's indirect cost reimbursements are cut, however, institutions may be forced to reduce the size of their research programmes. And if NIH awards fewer research project recipient grants, the institutions will have a smaller base for seeking indirect cost payments. To hear them talk now though, the two sides seem not yet to have realized that they have a common cause.

Deborah Shapley

Commercialization of research

Patent views

New York

Close on the heels of the closed meeting of five prominent university presidents at Pajaro Dunes, California, last month (Nature 8 April, p.479), the first of many other, open meetings was held last week in New York to discuss the growing issue of commercialism in academic research. This one was sponsored by the New York Bar Association's patent committee. Participants included Dr Steven Muller, president of Johns Hopkins University, Joshua Lederberg, president of Rockefeller University and A. Thomas Bartlett, president of the American Association of Universities (AAU).

The participants concurred with the conclusions of the Pajaro Dunes meeting concerning the problems of the commercial use of university-based genetic engineering research. In the words of Dr Muller, "the hazards to universities are manageable". It seemed clear at the meeting, though, that there can be no sweeping, general rules covering every campus and field of research in the nation, and that university policies will be built up step-by-step.

Those at the bar association meeting felt that university faculty would be well able to defer publication of key research in the open literature to satisfy patent needs of the commercial partners. Dr Lederberg noted that while such delays are "natural hindrances" to normal university activity, they should be acceptable to faculty so long as delays are reasonable. Dr Muller pointed out that Johns Hopkins recently passed regulations requiring university researchers to publish in the open literature and to discourage undue secrecy while faculty file for patents. The new regulations also place the burden of proof on faculty members who claim that a private invention has no relation to their university work.

One problem highlighted in the discussion was that European visitors to US campuses could pick up innovations in the course of seminars and talks with US scientists, go back to Europe and patent them, leaving the US academic inventor of the idea with no protection. This possibility arises because European law awards patents to the first to file for one, who may not necessarily be the inventor as in American law.

There was a call at the New York meeting for a re-examination of the relationship of professor to university and the traditional rights of university employers and their faculty employees, often ignored in faculty contracts. For example, can a university pressurize one of its professors to pursue a patent if he is reluctant to do so?

Dr Lederberg encouraged universities to help their faculty negotiate their outside contracts and to define extramural and intramural roles. He was concerned that many faculty are under-selling their services, and recommended that they charge six times their academic rate of pay for outside consultation. The universities can benefit, he said, by arranging that these fees be shared equally between a faculty and member and the institution.

Finally, several details of the best known university-industry agreements are now available to university presidents seeking to negotiate their own industry arrangements. University presidents seem to be making a practice of circulating current or draft agreements with industry among themselves for purposes of comparison.

Last year, Representative Albert J. Gore (Democrat, Tennessee) attacked the universities for their new industry ties and asked AAU to establish general guidelines on the matter. But the New York meeting seemed to confirm that such general guidelines are unlikely to emerge, or be seen as practical. As AAU president Bartlett said, "we are groping our way into the future".

Michael D. Stein

Dutch pharmaceuticals

New era vaccine

Waalre, The Netherlands

Intervet International, a Dutch veterinary pharmaceutical company, claims to have marketed the first vaccines produced through recombinant DNA technology. The Dutch are proud to have beaten two American companies — Norden Laboratories and Cetus — which recently announced that they were developing a vaccine against pig scours for introduction on to the market within two or three years. Intervet marketed two scour vaccines at the end of March.

The vaccines are intended to prevent infectious diarrhoea of pigs and calves, a major cause of which is the Escherichia coli bacterium. The bacterial components implicated in causing scour in pigs and calves are the adhesion factors K88 and K99 respectively. Combination of these factors with a special adjuvant was used by Intervet to produce a vaccine which, when given to the sow or cow, causes the animal to produce protective antibodies which are passed on to their offspring through the colostrum or first milk. In the past adhesion factors K88 and K99 have been purified from wild strains of bacteria isolated from the field. Using cloning techniques Intervet transferred the genes responsible for the production of the K88 and K99 into a laboratory strain of E. coli (K-12) which then produces a much greater quantity of antigen for the preparation of the vaccines.

The work to produce these strains started in 1979 in collaboration with the Dutch Institute of Health and was later transferred to the DNA research group within Intervet's mother-company Akzo Pharma, itself part of the Dutch chemical multinational Akzo. Casper Schuuring

Environmental research funding

Bleak outlook

The UK Natural Environment Research Council (NERC) is struggling against the somewhat cool attitude of the Minister of State for the Environment, Mr Michael Heseltine, towards science. The Chief Scientist's office at the Department of the Environment (DoE) is doing its best to protect NERC from budget cuts, but the task is not proving easy.

In fact, the department's attitude was so discouraging late last year that even NERC's chairman, the sanguine Sir Hermann Bondi, was beginning to be worried. When Bondi took up his post in October 1980 he defended those of Heseltine's cuts which were already affecting NERC, in the name of the "Rothschild principle" - that the customer (in this case DoE) pays for what it wants and the contractor (NERC) provides it. After more than a year's experience of DoE attitudes, however, Sir Hermann's line has softened a little: "I still believe in the Rothschild principle" he says, "but Rothschild assumed that there would be enlightened customers taking a long view".

The problem for NERC does not seem to lie in fundamental, or in applied research, but in the "strategic research" which is not obviously one or the other. A prime example is the geological survey of Great Britain, whose science may appear to be uninspiring, and which is applied only occasionally for major construction projects. In the long run, however, it provides a basic data set without which much other work would not be possible.

DoE's attitude has pushed NERC into finding more commercial contracts both in the United Kingdom, and abroad; but, say NERC staff, contracts in the United Kingdom cannot supplant the geological survey because they are too patchy, too specific and often entail a degree of commercial secrecy. Moreover, British firms are at least as short of cash as the government at present. Abroad, NERC have been seeking contracts actively, but without any major successes. Bondi would like to encourage this kind of expansion, but NERC is relatively new in the field and must compete with seasoned operators like the US Geological Survey and the Institute du Physique du Globe of Paris.

In the meantime, NERC staff must be supported — and the problem has been compounded by DoE delays in defining a programme of research for 1982-83. By early April, NERC would normally have expected to know DoE's requirements for the year. Worse, the council has been told not to spend money on the basis of DoE "letters of intent" until everything is signed and sealed, a further break with the usual practice.

However, there is some light at the end of the environmental tunnel. Recently NERC held a top level meeting with its three main

customer departments, those of energy, industry and environment and with the Central Policy Review Staff (the Cabinet's think tank) and there was a consensus that the problems lay with strategic research and long range planning.

NERC staff argued that if only they could be told the requirements of the departments well in advance, then the council could design a good basic and strategic research programme which would also yield the contractual results that the departments wanted. So the departments will work together to produce a five-year forward look for NERC, with the intention that this would be "rolled forward" annually thereafter.

Robert Walgate

NERC stays afloat

The British government has allowed the Natural Environment Research Council (NERC) one present: a new ship, specially designed for research, to replace Shackleton which is now a creaking 28 years old. The vessel is being bought on a loan from a merchant bank, and will cost NERC about £1 million a year for eight years out of its current £56 million budget.

The ship will be called *Charles Darwin*. It will not be ice strengthened, but will have a clear aft deck for equipment and will be able to tow over the stern, a big simplification over *Shackleton* (a converted container ship) which has to pull equipment from an A-frame over the starboard side. The *Charles Darwin* will be fitted for general research from marine biology to geophysics, and will provide the largest on-board laboratories of the NERC fleet. It should be delivered in 1984.

The research fleet will then include: Discovery, also a general purpose research ship, which must retire around 1989; the marine biological ships Frederick Russell and Challenger; and two ice-strengthened vessels. There are three other English research ships owned by the Ministry of Agriculture, Fisheries and Food (for fisheries research).

US recombinant DNA guidelines

Expected change

Washington

Continuing the trend towards relaxing controls on the newly commercial business of recombinant DNA research, the National Institutes of Health (NIH) has published revised guidelines that govern most US academic research and some industry research in the field. There are few surprises in the new guidelines; they follow the plan voted by NIH's Recombinant Advisory Committee (RAC) meeting in February (see Nature 4 February, p. 358).

The revised guidelines (published in the

Federal Register, 21 April and effective from that date) contain three important features. First, although there was pressure to abandon the present system of mandatory controls and change to a voluntary system, the mandatory system was retained. This means that organizations performing recombinant DNA research must still each have an institutional biosafety committee, and, for those doing work at P3 and P4 containment levels, a biological safety officer.

Second, no class of experiments is "prohibited" any longer. Of the five classes of experiments that were prohibited under the old guidelines, three are still required to undergo RAC review and NIH approval before initiation. These are drug resistance traits, toxin genes and deliberate release to the environment. Third, the section of the guidelines dealing with containment levels has been drastically simplified. Many experiments previously requiring NIH approval now require only the approval of the local biosafety committee.

In approving these guidelines, Richard M. Krause, Director of the National Institute of Allergy and Infectious Diseases of NIH, rejected a proposal for far greater relaxation of controls put forward last April by Dr Allan Campbell and Dr David Baltimore. These would have required a major overhaul of the guidelines. Apparently, Krause agrees with the majority of the members of RAC that such an overhaul is not appropriate — yet.

Deborah Shapley

UK nuclear power

Cracks no worry

New design criteria for the pressure vessel for a British pressurized water reactor (PWR) has been given the cautious approval of Britain's most distinguished PWR opponent, metallurgist Sir Alan Cottrell, now Master of Jesus College at the University of Cambridge.

The new criteria are the result of the work of the United Kingdom Atomic Energy Authority study group, headed by Sir Walter Marshall, on the integrity of PWR pressure vessels. The group last reported in 1976, but six years later the situation requires a fresh look. The design of the Sizewell PWR is going ahead; there have been changes in methods of steel manufacture; and there is now an accumulation of evidence and experience of stress corrosion cracking and of catastrophic failure tests.

In an open letter to Sir Walter Marshall, Sir Alan writes: "I would expect us to avoid the problems of localized cracking, underneath the stainless steel cladding, which the French have experienced in some of their vessels".

He adds "Whether we shall be entirely clear of the problems of metal fatigue and crack growth associated with corrosion is less certain, but the evidence now is far better than it was in 1976 and I agree with the report and you that it shows that crack growth rates are — or can be, if the quality of the metal and the water chemistry are closely controlled — much lower than appeared to be the case in 1976".

The report estimates that a hidden crack in a pressure vessel would have to be at least 75 mm in "height" to initiate crack propagation under normal operating conditions. ("Height" is defined in the report to be the vertical distance from the surface between the nearest and furthest surfaces of the crack.) The best estimates of stress corrosion crack growth indicate that cracks of a starting height of, say, 50 mm, would grow less than 10 mm during service.

The report also describes methods by which cracks can be prevented and monitored during the manufacture of the pressure vessel and during service, and in these respects, says Cottrell, the report goes much farther than its predecessor.

However, in the end Sir Alan remains very cautious. "To summarize", he writes "provided the recommendations in the report are all accepted and fully applied in practice; and in particular that arrangements are made to keep all parts of the pressure vessel in the 'upper-shelf' range of fracture toughness at all times; and arrangements are made to ensure that the vessel is free at all times from cracks, down to a size at which growth becomes insignificant under all conditions; then a PWR pressure vessel subject to all these conditions will have a high integrity and reliability in service". Robert Walgate

University redundancies

Now the crunch

British universities seem well on the way to solving their budgetary problems by persuading tenured academics to opt for early retirement. Hundreds of applications to retire early from members of teaching staffs have already been accepted. But many universities are left with the nagging doubt that the terms agreed with academics willing to go early may not qualify for reimbursement under the scheme the University Grants Committee (UGC) has now made public.

In February the UGC confirmed that it would reimburse in full the cost of payments to staff made redundant because of cuts in the government grant. In the UGC's letter to the universities, the term "redundancy" covered early retirement. For academic staff over 50 the approved terms are those of the Premature Retirement Compensation Scheme (PRCS) of the University Superannuation Scheme (USS) introduced in 1979. An eligible employee receives the USS pension and any accrued benefits plus a compensatory pension and lump sum payment calculated in "Scheme" years. The cost to the university

is the cost of paying into the pension fund this extra benefit.

The scheme allows academic staff below the age of 50 — not covered by PRCS — to receive a deferred pension, a lump sum payment of one month's pay for every year of service, and one month's pay for every year of service in excess of five years or after their 30th birthday, whichever is the greatest. Payments of up to £55,000 plus pension rights could be made to a redundant 50 year old. The scheme is essentially that put forward by the Committee of Vice-Chancellors and Principals except that the pensions of staff aged 50–55 who opt for early retirement will not be index-linked.

Conditions for reimbursement are stringent. The PRCS was not formulated as a redundancy compensation scheme and universities offering enhanced terms will not be repaid. Terms negotiated before 5 February will, however, be supported but only up to the limit of the government-approved scheme. Staff cuts must also be "consistent with academic plans" — UGC may refuse to pay out for redundancies which do not form part of a comprehensive economy drive.

Volunteers for redundancy schemes will have a good chance of being re-engaged for part-time teaching. The UGC will not reimburse for staff who are to be replaced, but recognizes the need to make a gradual adjustment to staff losses. The cost of taking back staff on a part-time basis will be reimbursed only if contracts are signed before the end of the academic year 1984–85 and do not extend beyond three years.

How are the universities coping with the cuts? Salford is hoping to lose 500 staff members (120-145 of them academic) by the end of the academic year 1984-85. Twelve of its 45 professors are to leave by September, and in order to maintain a balance of experience and age distribution the university is keen to get voluntary severances from younger staff.

Leeds was one of the first universities to respond to government cuts by planning staff losses and it is hoping that its enhanced retirement scheme will make it eligible for compensation. Of the 145 applicants, 95 will have been accepted for early retirement. The bulk of staff losses were accepted under the first stage of its economy plan — a 6 per cent cut in expenditure by the end of the academic year 1982–83, but cuts to its budget and the repercussions of the government's overseas students policy have now forced a 10 per cent reduction in expenditure.

At the University of Cambridge there are doubts as to whether the university's plans will be eligible for UGC compensation. Forty academics have responded to an appeal to take early retirement. A scheme providing enhanced compensation was offered to university staff aged 58 to 60 after its approval by the Council of the Senate in February. The university hopes to attract 100 volunteers in a drive to knock £2 million a year from its expenditure by

July 1984. The university has had to offer enhanced terms because, compared with the age of 65 on which the PRCS scheme is based. Cambridge academics have tenure until 67. Eligible applicants will receive a full pension plus a lump sum calculated on the basis of the pension they would have received at normal retirement. The university is pleased with the response so far.

Jane Wynn

UK biotechnology

A start in sight

The British government has at last fulfilled its promise, made more than a year ago, to establish machinery for coordinating the national effort in biotechnology. Last wek, Kenneth Baker, the minister for information technology, announced the setting up of an interdepartmental committee on biotechnology under the chairmanship of Dr Ron Coleman, the recently-appointed Government Chemist.

Dr Coleman's chairmanship may seem rather unusual, chiefly because the Laboratory of the Government Chemist of which he is head, has traditionally followed a fairly narrow remit of providing analytical services to the public sector. But Dr Coleman's desire to broaden its scope has persuaded the Department of Industry to make the laboratory responsible for the department's interest in biotechnology. The new committee's membership includes all those government departments with an interest in biotechnology together with the medical, agricultural and science and engineering research councils, the British Technology Group, the Public Health Laboratory Service and the Centre for Applied Microbiology and Research, Porton Down.

The chief task of the committee, which will have no money of its own, will be to coordinate its members' separate interests. One question close to its heart will be how to transfer the results of biotechnological research into industry. That will involve paying special attention to the role of the Biotechnology Directorate, recently set up by the Science and Engineering Research Council, and the British Technology Group, whose task it is to exploit academic research in industry.

The committee's other main objectives are to foster international collaboration in biotechnology and to look for ways of ensuring the continued existence of culture collections, which at present suffer from being funded from a variety of different sources. The committee, which has before it a study commissioned by the industry department on the organization and funding of culture collections, will be looking for one government body to take over their administration and may recommend changes to the fees now charge for the services provided by the collections.

Judy Redfearn

French universities

Into reverse

One of the first steps towards undoing the authoritarian reforms of the French university system introduced by the previous minister of universities, Alice Saunier-Séîté, has just been taken. Students have been allowed back onto the university councils, the senior administrative councils of the universities.

The reform would be radical — if the students were radical. But the present atmosphere is nothing like that of 1968. The new student representatives are conservative and unreforming, say university staff. For example, in one recent meeting in the University of Paris, students warmly approved a proposal for more examinations and greater selectivity — something which would have been anathema fourteen years ago.

The underlying question is not what the students will do, but where the government will stop in this new attempt to restructure French higher education. M. Alain Savary, the minister for national education (which now includes the universities and grandes écoles), has some plans which are causing shivers in the French establishment. Not least among them is that the classes préparatoires, the usually private courses that prepare students for entry into the prestigious grandes écoles, should be put into the hands of the universities.

This would mean that the brightest of French schoolchildren, who normally pass through the classes préparatoires on their way to the grandes écoles (and thence to management and government administration), would be exposed to a university environment. There they would learn that the universities were not the second class establishments that some suppose, the argument goes, and some might even choose to stay and read for university degrees.

However, such proposals are too radical for the *grandes écoles*, who are resisting them so strongly — and are so well represented in government — that the political will at the ministry of education is beginning to falter.

For researchers, the situation is even more complicated. There is a conflict afoot between the minister for research and technology, Jean-Pierre Chevenement, and M. Savary. Chevenement has battled long and hard for control of all government spending on civil research and development. Much of the research is done in the university environment, in laboratories which are now funded by Chevenement's ministry. In general, the researchers in these laboratories (such as those of the Centre Nationale de la Recherche Scientifique) do little formal teaching in the university (although they help to train researchers through seminars and research degrees). Chevenement is believed to want more of the teaching to be done by his researchers,

to improve the training of scientists; but this would remove some of the university teachers' higher level and more interesting work. So university opposition is growing to these moves, and Savary inevitably represents one side and Chevènement the other.

How this battle will develop is anyone's guess, but it could easily lead to another deadlock, when the promised higher education reforms would merely tinker with the system, rather than removing its two main faults: the class divisions between the grandes écoles and the universities, and between the researchers with formal teaching responsibilities and those without.

Robert Walgate

Moves on energy

The French government is showing every sign of pain in its attempts to embrace the environmental movement. In the latest agonies, the council of wise men which advises the Minister of Industry on nuclear safety has been reformed to include a few token nuclear opponents; and the promised new agency for renewable energies is to be set up, but shorn of FF 170 million.

The safety council is the Conseil Supérieur de la Sûrété Nucléaire, presided over by Professor Louis Néel, an ex-director of the French atomic energy agency laboratories in Grenoble. Néel is described by his opponents as "totally pro-nuclear" and, indeed, his council has met only three times in the past seven years, giving the previous government a pretty clear run on safety matters. Under the new arrangement, Néel stays as president of the council, but it is to meet more frequently (two or three times a year) — and there will be a few cuckoos in the nest.

These come in part from the addition of a further seven experts in the council, including a science journalist, M. Serge Berg of Agence France Presse. The biggest change, however, is the inclusion of trade union representatives on the council for the first time, among them Bernard Laponche, a prominent nuclear critic from the more environmentally inclined of the two major unions, CFDT. So far Laponche is not impressed. The council is still not complete and no date or programme has been set for its first meeting.

As for the renewable energy agency, Agence Nationale pour la Maitrise de l'Energie (ANME), this will regroup the existing solar energy and energy conservation agencies, and control their budgets; but present indications are that its budget will be less than the sum of its predecessors. The cut in budget is largely the result of a deal to buy Algerian natural gas – the energy conservation agency loses FF 170 million (£17 million) to help pay for the gas.

Robert Walgate

Radioactive waste in India

Looking to glass

New Delhi

The world's second commercial plant for the immobilization of radioactive wastes from nuclear power generating stations is soon to be built at Tarapur, in the western Indian state of Maharashtra. The first such plant is already in operation at Marcoule in France.

The new plant is being set up in the wake of increasing concern in India that radioactive waste from nuclear power installations might damage the health of the people — although there has been no scientific evidence of such damage so far from India's two nuclear power plants at Tarapur and Kota in Rajasthan state.

The radioactive waste immobilization method being adopted at Tarapur is a semicontinuous process which incorporates the oxides of high level waste from fuel reprocessing into borosilicate glassy matrices. The nominal throughput of the plant is about 25 litres of waste an hour, equivalent to the immobilization of wastes produced from reprocessing about one tonne of spent fuel a day. The plant would produce about 125 kilogrammes of glass a day, which is to be stored in stainless steel containers.

The process was developed in India and differs in many respects from the British and French techniques. The wastes are first calcined with glass fines in a process canister and then melted into a glass form. After sufficient "soaking" to help achieve homogeneity, the glass is cast into storage canisters — which can be inexpensive containers as the glass waste is more easily stored than liquids.

The storage units are stored in an engineered near-surface facility where they undergo continuous natural draught cooling to dissipate the decay heat under constant surveillance. The plant is designed to provide cooling for 20 to 30 years and incorporates facilities for establishing the characteristics of the waste products when the time comes for ultimate disposal.

The ultimate disposal medium has not yet been chosen, although current research favours a hard rock formation such as gneisses or granite.

The high-level radioactive wastes arise from the reprocessing of spent uranium fuel for the extraction of plutonium. The Atomic Energy Commission now proposes to build a chain of reprocessing plants in the country near the sites of future nuclear power stations. The proposal follows the United States' refusal to provide enriched uranium for the Tarapur nuclear power plant unless India meets conditions specified by the United States in the name of nuclear non-proliferation. Plutonium extracted by the reprocessing of spent fuel would therefore substitute for supplies of enriched uranium withheld in this way.

Sunil Saraf

CORRESPONDENCE

Creative engineers

Sir — Darnbrough (*Nature* 18 March, p.192) and Austen (*Nature* 25 March, p.284) both miss, or intentionally obfuscate, my analogy about the evolution of aeroplanes and biological evolution. Both processes lead from the simple to the highly complex. Hoyle and Wickramasinghe, not I, chose the aeroplane simile and implied that evolutionists believe that "higher life forms evolved by chance", and that a gene "might be chosen from 10²⁰ nucleotide sequences of the appropriate length". This implies that large complex proteins appear without any intermediate

Actually, proteins are thought to have evolved by the joining together of short polypeptides, as pointed out by me (Molecules and Evolution, Columbia University Press, pp. 222-229, 1960), and further explained by Rout (Nature 18 March, p.192). Yeas has given striking examples of this process in "periodic" proteins such as collagen and keratin (J. molec. Evol. 2, 17; 1972). Lengthening was probably by duplication and recombination in DNA, followed by an accumulation of point mutations.

Obviously the evolution of aeroplanes, completed in a few years, was a product of the creative genius of engineers. The point was that the Boeing 747 did not suddenly appear on the scene. In contrast, biological evolution took millions of times as long, and was impelled by slow, insensate natural phenomena. Austen says that "without a Creator, atoms and molecules, which He created, are still atoms and molecules". How could atoms and molecules exist without a Creator if He created them?

THOMAS H. JUKES

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A thermal history

Sir — Many letters in recent issues of *Nature* show little unanimity about the mechanisms or rate of evolution. There may, however, be agreement that organisms do not adapt to, or evolve for, life in non-existent environments: nor do they retain capacities needed in an environment in which they no longer live.

In the course of their mainly reasonable discussion on the probable thermal history of Earth, while exposed to a steadily increasing rate of inflow of solar energy, Lovelock and Whitfield (Nature 296, 561; 1982) quote the observation that 50°C is the "critical upper limit for most life" as a factor supporting their thesis that the overall temperature never fluctuated greatly from today's values. That particular piece of evidence is not very cogent. Hot environments, such as hot springs and submarine spreading centres, are small and, from an evolutionary and geological standpoint, evanescent. Circumstances do not now exist that favour the evolution of organisms adapted to life at high temperatures, nor the persistence of that ability if it existed earlier.

The fossil record tells us nothing about the thermal preferences of very early organisms.

Biological potentialities should not be underrated. Enzymes vary in thermostability so, if granted several million years of undisturbed occupation of an extensive hot site, there is good reason to expect the emergence of proteins more robust than those with which we are familiar. It is not just a coincidence that the boiling point of water under today's atmospheric pressure is the limit beyond which no organisms are known to be able to live, and that very few organisms can approach that temperature. There would be no advantage in the ability to exceed that temperature - or in many organisms adapting to it. And proteins are not the only conceivable vehicles for quasi-enzyme activity. Had there been a prolonged period when the overall temperature was greater than it is now, there is no reason to assume that organisms adapted to it could not have existed. Recognition of this possibility extends the scope of discussion about the origins of life. N. W. PIRIE

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Not unilaterally

SIR — Though you published an editorial (*Nature* 1 April, p.380) commenting on the three letters contained in that issue replying to the editorial of 18 February (p.542) criticizing the use of professional reputation to give credibility as it were through the backdoor, to causes like disarmament, you failed to notice the essential sophism of the points in the letters. And as this sophism is of much wider consequence than the matters addressed in these letters, affecting the whole popular movement of opinion on a subject of great importance, it deserves attention and debate.

The error is seen in the tendency to deduce from the articulated description of the horrors of nuclear war, the wrongness of, and hence the desirability to prohibit, nuclear arms. It was not felt by any of your correspondents necessary, beyond showing how awful the results of nuclear war are, to argue that disarmament has any relevance to diminishing the risks of war.

It seems to me that those who in supporting disarmament say that they are primarily concerned to achieve a wider public awareness of the consequences of nuclear war, are in fact advocating response rather than consideration. They imply by arguing how wrong nuclear wars are, that the obvious conclusion is that to prepare for the eventuality of such a war is also wrong. To argue like this is to take advantage of the association of ideas in the mind, but to evade the rational consideration of what causes war.

Although it is not my intention to argue for nuclear weapons, only to point out that the greater salience of an idea does not excuse a failure to demonstrate the validity of an argument, it is important to remember that wars are initiated in the expectation, however misconceived, that winning them is possible. Nuclear weapons as presently shared between the superpowers do not allow such misconceptions, though after unilateral disarmament one fears they could.

London NW3, UK

J. R. SKOYLES

Propaganda war

SIR — Your leading article of 1 April (p.380) headed "Professional propaganda" complains that professional groups, such as physicians who state that the threat of nuclear war currently presents the most urgent challenge to preventive medicine, do not at the same time offer alternative political and military solutions to the problems of arms control and defence. I suspect that if they were to try to do so you would censure them for making pronouncements on subjects beyond their expertise.

You would, however, be mistaken to presume that those who take the step of joining organizations such as Physicians for Social Responsibility in the United States or the Medical Campaign against Nuclear Weapons in the United Kingdom are politically ignorant. It is because they know enough to be unconvinced by assurances that nuclear weapons are necessary and for the best that they try to bring home to the public and to politicians the dangers of regarding such weapons as acceptable instruments of policy. Similar concerns also stir physicians in other countries, both East and West. At a congress held in Cambridge recently by International Physicians for the Prevention of Nuclear War (an arrogant title, perhaps, but not inaccurate) some two hundred eminent participants from 31 countries were unanimous in concluding that nuclear war - whether intended or resulting from tragic accident - would be catastrophic for any countries involved and might have dire consequences for humankind, that the medical services could have only a marginal effect in mitigating the suffering of immediate survivors, and that it was a continuing duty to bring this home to the public and to politicians in their own countries.

The congress agreed some carefully prepared statements, among them letters to President Reagan and Chairman Brezhnev which included the following outright recommendations for political action:

"We believe that action must be taken now to prevent nuclear war and to relieve the consequences of the arms race. We urge that as a first step, the nuclear powers cease all further production, testing, and deployment of nuclear weapons and their delivery systems. This should be accompanied by mutually acceptable methods of verification. We further urge that all nuclear powers unequivocally renounce the use of such weapons, and agree to prevent their introduction into any conflict. We advocate effective bilateral and multilateral negotiations on the limitation, reduction, and ultimate elimination of nuclear weapons.

We are aware that negotiations have been in progress for many years with essentially no apparent effect on the arms race. While we welcome any initiative by either side to reduce its stock of nuclear weapons, we recognize that they are likely to be eliminated only by negotiation. We must again urge that such negotiations be pursued seriously and continuously until they succeed."

J. H. HUMPHREY

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Britain's resources of coal and spent uranium fuel

Robert Olby*

THE United Kingdom is often described as a favoured nation in an increasingly energy-hungry world - possessing riches of coal, and also North Sea oil and gas and an abundance of spent fuel from nuclear reactors. Before the OPEC oil price rise of 1973 it was generally accepted that dependence on coal should continue to decline in favour of oil and nuclear power. The imposition of a fuel tax on oil in 1961 marked an attempt to slow down the rate of oil substitution, but nevertheless between 1959 and 1973 oil consumption more than doubled, and by 1973, 3 per cent of total primary energy was supplied by nuclear power. The action of OPEC not only strengthened the argument for nuclear power based on its contribution to national security but within the National Coal Board it brought to the fore the already active concern to reassess the future of

In the board's Plan for Coal (1974), investment of £1,400 million over the following decade was proposed, £8 million, of which was to be spent on an intensified programme of exploration to decide on the best sites for future development. Three years later, this expansionist mood became apparent to all with the announcement of the board's revised estimates of the United Kingdom's "physically recoverable" reserves. To a British Association audience the then-chief geologist to the board, A. Michael Clarke, declared that economics and a lack of sufficient physically recoverable reserves of coal were not the sole determinants of the choice between coal and nuclear future bulk energy supplies for Britain and Europe. Just because the era of cheap oil had taken an unnecessary slice from the life of UK coal reserves, it was not the case that in the long-term we lacked adequate reserves. To go nuclear, he declared, was not therefore inevitable.

The United Kingdom's reserves, which the World Energy Conference Survey of Energy Resources for the reference year 1975 (published 1977) had shown as 3.887×10^9 tonnes, were now claimed by the board to be 45×10^9 tonnes. Small wonder that this apparent dramatic change caused adverse comment from the Institution of Geological Sciences (IGS), letters in The Times and a tart editorial in Nature.

In 1975 Sir John Hill, chairman of the Atomic Energy Authority, drew attention to the potential energy source available in spent uranium fuel. With the fast reactor, he claimed, Britain's spent fuel could yield

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as much as 50×10^9 tonnes of coal, more, he declared than Britain's total coal reserves which, according to the National Coal Board in 1969 were about 20×10^9 tonnes. In 1976 Sir John's colleague T.N. Marsham gave the more conservative coal equivalent of stored spent fuel as 20×10^9 tonnes, which Sir John later doubled to 40×10^9 tonnes a year. Between these two values came the Coal Board's new estimate of Britain's coal reserves at a little more than twice T.N. Marsham's figure.

rate of expansion of coal extraction of the first half of that century (800 per cent). Such a drain on the country's "life-blood" would exhaust its resources by AD 2034. On the other hand, if, as Edward Hull believed, the coal industry would be incapable of raising more than 100 million tons per annum, the nation still had eight centuries of supplies. Meanwhile he looked to science to show how we could win energy from "the light and heat that is everywhere around us". Appealing to God's provi-

During the 1970s estimates of the United Kingdom's reserves of coal and spent fuel from nuclear reactors, provided by the National Coal Board and Atomic Energy Authority respectively, have tended to steadily increase. It is tempting to infer a connection between these estimates and the efforts of the NCB and AEA to promote their claims on government finance, by each predicting as long-lived a future secure energy source as its rival. This article follows the history of the various estimates and attempts to assess their credibility.

Dwindling resource

Although the long-term future of coal as the United Kingdom's chief energy source was a matter for concern after the Second World War, the subject had long been debated. In 1789 John Williams discussed the "limited quantity of coal in Britain" in his book Natural History of the Mineral Kingdom, and in the 1810 edition edited by James Miller, Macnab's 1792 estimate of 360 years' supply was given. This figure related only to the coalfields of Northumberland and Durham and the extraction rates of that period. In the nineteenth century the Oxford geologist, William Buckland, urged upon the parliamentary committees of 1830 and 1835 the need to conserve coal. In 1830 the old sea-coal tax had been abolished, but twelve years later Sir Robert Peel put a tariff on all exported coal.

The subject of coal resources came before Parliament once again when the Commercial Treaty with France was debated in 1860. According to Article 11, no duty was to be levied upon coal exported to France and other nations with whom the United Kingdom was at peace. It was the discussion of this treaty which caused Edward Hull, member of the Geological Survey, to write The Coalfields of Britain (1860) in which he took a sanguine view. Introducing the mining limit of a depth of 4,000 feet he reckoned England and Wales had just under 59×109 tons, enough for about a thousand years. In the second edition of 1862 Hull included the Scottish coalfields which raised his estimate to 79.8×109 tons. Hull recoiled from the implications of continuing indefinitely the dential character he assured his readers: "nor can we suppose that any part of the Creator's universe has been regulated on so short-sighted a plan, that it shall become disorganized because some of the elements necessary to its economy have failed".

The optimism of Edward Hull was matched by the stern realism of the economist and statistician, W. Stanley Jevons. In his celebrated work, The Coal Ouestion (1865) he calculated the duration of British coal on the basis of Hull's estimate of reserves and the extrapolation into the future of the historic trend in coal consumption, which had been 3.4% per annum over the decade 1851-61. His conclusion was that: "Rather more than a century of our present progress would exhaust our mines to the depth of 4,000 feet . . . " Jevons was widely misunderstood as predicting exhaustion around the year 1975, whereas he was really trying to show that the nation's industrial expansion could not continue for long at the rate then current. Whilst there was still plentiful cheap coal attempts should be made to reduce the national debt, introduce a general system of education, and impose a far more general restriction on child labour. Such tasks would be more difficult to achieve when other countries reached and surpassed the output of Britain's mines, becoming more competitive in world markets (by 1967 US pithead prices were half those of Britain). Although Jevons foresaw this situation he could not, as a free-trader, recommend the imposition of an export tariff on coal. Therefore it was clear to him that the cost of coal extraction was a decisive factor in determining the proportion of Britain's total coal resources which would be extracted. In the future we might, indeed, be able to mine coal at greater depths than at present, but only if the price was competitive in world markets. There was no question of the physical exhaustion of our coalfields—"though we may some day have to pay dear for fuel; it will never be positively wanting", he explained in the second edition of *The Coal Question*. Here Jevons was expressing what later became a major feature of the distinction between reserves and resources, the history of which is recounted below.

Jevons drove his point home by a demonstration of the unique character of coal. In a remarkable chapter on subtitutes in the third edition of his book (1906), he discounted sunlight, peat, hot springs, water and wind power, the tides. petroleum, hydroelectricity and hydrogen produced by the electrolysis of water. He found it absurd to picture the 7,308 horses or 1,000 large windmills which would be required to power a modern factory like the great Dowlais ironworks, and he pointed to the fallacy of considering electricity as a source of self-creating power. The chief targets of this critique were the optimists, Dionysius Lardner, Charles Babbage and Edward Hull.

Sir John Herschel applauded Jevons. "Such a work as yours", he wrote, "has long been wanted to dissipate completely the delusion which so large a majority of our countrymen labour under, of the 'inexhaustibility of our mineral resources' etc. and the 'probability amounting to certainty' that science will ere long put us in possession of a substitute for coal . . . after this let no man plead ignorance and say 'who would have thought it'." Herschel went on to describe his favourite notion of using the tides by running pipes under the sea to a distance of 1,000 feet around 1,570 miles of coastline from Berwick-on-Tweed to the Solway Firth. With a 6-foot tidal fluctuation he reckoned this vast construction would supply only one million tons of coal equivalent — one fifth of London's annual consumption or enough to keep ten Great Easterns constantly under steam.

By the summer of 1866, Jevons had become a newspaper celebrity. Gladstone wrote to him, J.S. Mill quoted him and Queen Victoria appointed a Royal Commission on coal chaired by the Duke of Argyll. In its report five years later, this commission estimated the amount of coal which "may reasonably be expected to be available for use" as 90.2×10^9 tons in ascertained coalfields, plus a possible 56.3×10^9 tons elsewhere. The resulting total recoverable resource figure of $\sim 146 \times 10^9$ tons within a depth of 4,000 feet appears to have been accepted.

The commissioners were sceptical of making projections of future demand based on historic trends; they doubted that Britain's coal would ever be absolutely exhausted, but the country would lose its

advantageous position in regard to coal supplies, coal imports would become the rule rather than the exception. Ominously the report concluded with the sentence: "But it may well be doubted whether the manufacturing supremacy of this kingdom can be maintained after the importation of coal has become a necessity." These views were not substantially revised by the Royal Commission of 1901, nor by anyone else.

Like their 1871 predecessors, the 1901 commissioners misunderstood Jevons. They arrived at an estimate of total available coal to 4,000 feet of nearly 142×10^9 tons, 40.7×10^9 tons being in "unproved" coalfields. The approximate agreement between the two commissions' estimates, despite the quantity of coal extracted in the interval, was due in Jevons' son, Herbert's, opinion "to the well known law that a number of unbiassed errors tend to cancel one another". Nevertheless it consolidated a growing consensus that Britain had at least 140 × 109 tons of which the proportion in "proved" coalfields was increasing due to continued exploration. Conversely, the consensus was developing that as more of the best and most accessible coal was removed, the remainder would be increasingly difficult and expensive to mine. Therefore, the 1901 commission declared, the rate of increase of extraction, "will soon be checked by natural causes" so that there was "no present necessity to restrict artificially the export of coal in order to conserve it for our home supply".

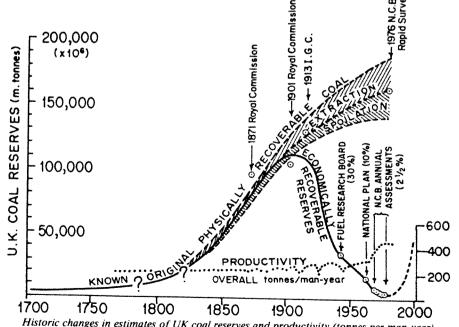
In the inter-war period, the coal industry laboured under many difficulties, the chief of which was falling demand. By the time of nationalization in 1947 it was in a parlous state, having suffered from many years of financial stringency and lack of investment. Despite extensive mecha-

nization output had not grown as it had in continental mines. This was chiefly due to the winding and undulating character of the underground lay-outs ("in-seam mining") in Britain in contrast to the straight roads which were driven through the strata on the continent ("horizon mining"). The latter layout was easier to mechanize throughout. In Britain the work of the coal-filler at the end of the conveyor belt remained unmechanized and constituted the bottle neck in the system until the 1960s.

Although Britain did not suffer so acutely after the Second World War as other European countries from coal shortages, demand was depressed by the system of allocation to consumers and there were frequent electricity cuts. Even allowing for the growth of the oil industry with Marshall Aid a long-term reorganization of the coal industry would be required to expand production. Such anticipated expansion did continue until 1958 when the threat of cheap oil began to bite into coal's traditional markets confounding the prophets of its future demand.

In such difficult circumstances mines were being closed at a rate of 40 a year, and the economically workable reserves were written off (depleted) at an alarming rate. The result is seen in the figure below.

The value of 3.87×10^9 tonnes appeared in the 1974 World Energy Conference Survey of Energy Resources alongside a figure for total coal reserves of 99×10^9 tonnes and almost 163×10^9 tonnes for total resources. It did not escape the notice of the conference report that the United Kingdom's recovery percentage was thus only 4 per cent in contrast to West Germany's 40 per cent. On his return from



Historic changes in estimates of UK coal reserves and productivity (tonnes per man-year). Adapted from A.M. Clarke in Australia's Mineral Sources (eds G.M. Philip & K.L. Williams, Sidney, 1978).

the conference Leslie Grainger, the National Coal Board's Member for Science, pointed out the absurdity of these data to the board.

A hint of what had happened can be found in the World Energy Conference Survey for reference year 1975. Although total reserves were now nearly 101×10^9 tonnes, economically recoverable reserves were still only 3.887 × 109 tonnes. From a footnote we learn that this figure concerned reserves at existing collieries and opencast sites (what are usually described as "operating reserves"). A second footnote tells us that the National Coal Board's "alternative" estimate for economically recoverable reserves (not restricted to those with access from existing mines and by local marginal costs) was 45×10^9 tonnes.

Resource terminology

Was this confusion merely a product of the semantics of resource estimates or of some deeper issues? Clearly there is still not a consensus over the former if only because different resources tend to be suited to different terminological rules and the needs of the smaller commercial mining companies are not the same as those of a nationalized industry. The roots of a precise terminology can be seen in Herbert Hoover's little classic, The Principles of Mining (1909), where the tripartite division of mineral ores into proved, probable and prospective was recommended. Four years later the staff of the Canadian Geological Survey, in their task of assessing world coal resources for the twelfth International Geological Congress, asked for returns to be classified into two groups (above and below 4,000 foot depth) and these to be subdivided into actual, probable and possible. These terms, or their equivalents, were used by mining engineers and mining geologists in the preparation of expert advise on the likely profitability of mining ventures. They expressed the level of confidence to be placed on the extent and availability of the resources in the mine, and were intended to protect the investor from exposure to fraudulent claims made on behalf of unsound commercial ventures. Therefore they referred to existing technical and economic conditions.

During the Second World War the United States government, concerned about their mineral resources, held Senate hearings on the subject at which the Bureau of Mines and the Geological Survey (USBM/USGS) proposed a new tripartite definition of reserves as measured, indicated, and inferred, each division representing defined levels of reliability, the highest being the ± 20 per cent of measured reserves. The long-term needs of such national assessments led S.G. Lasky in 1949 to make an important distinction between reserves and resources, two terms hitherto used interchangeably. Whereas reserves (measured, indicated and inferred)

were known to exist and had "aspects of usability within a practical limit of time and within a specified set of economic and technologic conditions", resources comprised "all materials in the ground, discovered or undiscovered, usable at present or not, . . . considered within the context of all factors . . . that may influence its conversion into a "reserve", and . . . that enter into prediction or opinion as to possible future usability". This distinction was recognized in Blondel and Lasky's 1956 equation:

Resources = Reserves

- + Marginal resources
- + Submarginal resources
- + Latent resources

It has become increasingly recognized internationally, and has influenced the World Energy Conference classification whose 1974 Survey definitions were:

- Total resources: "The total quantities available in the earth that may be successfully exploited and used by man within the foreseeable future."
- Total known reserves-in-place: "The corresponding fraction of resources that have been carefully measured and assessed as being exploitable in a particular nation or region under present local economic conditions using available technology."
- Recoverable reserves: "That fraction of reserves-in-place that can be recovered under the above economic and technical limits."
- Additional resources: "All other classifications with a lower degree of geologic certainty as to their existence than those indicated as known."

Of this last category the 1980 Survey remarked that they:

embrace all resources, in addition to proved reserves, that are of at least foreseeable economic interest. The estimates provided for additional resources reflect, if not certainty about the existence of the entire quantities reported, at least a reasonable level of confidence. Resources whose existence is entirely speculative are not included.

What, then, is the meaning to be attached to the United Kingdom's 1977 figures of (in tonnes):

Total reserves	100×10^{9}
Economically recoverable	45
reserves	
Additional resources	45
Total resources	190

The National Coal Board maintains that within 1.2 km depth and in seams over 60 cm thick there are about 190×10^9 tonnes of coal, of which some 100×10^9 tonnes are well ascertained but no figure has been given for the confidence level. These estimates are in line with the general trend of the Royal Commissions of 1871 and 1901. The figure of 45×10^9 tonnes for economically recoverable reserves shows the recognition of the fact that recovery of what is known to be in the ground has fallen from the nineteenth-century level of between 70 and 80 per cent to about 50 per

cent. This fall, due to both economic factors and to mechanization, could in Clarke's opinion be reversed under high oil prices and a vigorous programme of coal exploration. Over the next decade K. Moses thought the figure for economically recoverable reserves might well rise to 50 or even 60×10^9 tonnes. Clarke suggested the possibility of returning to the older recovery percentages which would push the figure up to 90×10^9 tonnes. Before the oil price rise of 1973, George Armstrong, then chief geologist to the National Coal Board assessed the current recovery of reserves at about 25 per cent, but he clearly anticipated a major change in the industry given increased investment. With capital, he explained, "access could be made to the best seams in the vast extentions of our existing coalfields", and we should see "for the first time since the latter part of the nineteenth century . . . an increase in the reserves of coal in Britain which can be considered as economically workable". He never denied the 200×10^9 tonnes total UK resources but considered this figure of "only academic interest" and "grossly erroneous as an estimate of economically workable reserves". It was right that under stricter rules of resource estimation the old UK figures, like the early American ones, should have been reduced. It was not that some of this coal had never existed, or had vanished since 1900, but that the onus was put on the coal industry and the nation to support the exploratory research and technical development needed to bring more and more of the resources into the reserves, and thus afford a clearer picture of the future potential of our coalfields.

Nevertheless, it should be stated that there is not yet a consensus between geologists within and without the coal industry as to how much of the coal in the UK's abandoned fields remain accessible for future extraction.

Towards atomic power

The recognition of the industrial advantage to be reaped by the nation which leads the world in the exploitation of a new source of power lay behind the public and governmental enthusiasm for exploiting the atom. Radioactivity was considered as a possible novel source of power long ago in the Times Literary Supplement for 17 July 1903. Radium was considered amongst those "gigantic possibilities, which are not probabilities at all yet . . . ". Frederick Soddy, who had "ghosted" the article, pictured how much energy radium would yield if its rate of disintegration could be speeded up. Rutherford, the article reported, "has calculated from his own experiments and those of Curie that the energy stored up in one gramme of radium is sufficient to raise 500 tons a mile high. An ounce would therefore suffice to drive a 50 horsepower motor car at the rate of 30 miles per hour round the world". If it could be achieved with radium if could be achieved with uranium and thorium. "Our

fathers busied themselves with speculating what would become of us when the world's supply of coal was exhausted. A single step of science is needed for that problem to be answered in a manner beyond the dreams even of the scientific novelist."

We all know that these prophetic words were fulfilled (for uranium not radium) when in 1939 Lise Meitner and Otto Frisch interpreted the experimental results of nuclear fission; and Halban, Joliot and Kowarski, Fermi and others discussed the possibility that it might be accompanied by a chain reaction. That year was not only the annus mirabilis of nuclear fission, it also marked the first attempt to calculate the potential electricity output of a power station run from a "uranium boiler". S. Flügge calculated in Die Naturwissenschaften that complete fission of one cubic metre of uranium oxide powder would yield 7×10^{10} kWh of electricity, sufficient to replace the entire output of Germany's power stations running on middle-German brown coal for 11 years.

Comparisons such as Flügge's have been made repeatedly since 1939 in order to give some conception of the contrast in the concentration of energy in fissionable fuels as compared with fossil fuels. Hitherto the largest yield from a nuclear transformation had been the 22.2 MeV from the conversion of lithium (6Li) into alpha particles (4He). But when M.C. Henderson measured the energy yield from uranium in 1939 it was a staggering 175 meV (theoretical value, 198 MeV). (The beta decay of radioactive fission products subsequent to fission of 235U is 21 MeV, and has to be subtracted from the 198 MeV.) The table below illustrates the million-fold increase in yield per atom when we go from combustion to fission.

Theoretical energy yields per atom

12C	Combusion	4.17 ev		
²²⁶ Ra	Fission	4.79 MeV		
235U	Fission	198 MeV		

When we compare uranium and carbon weight for weight the ratio is approximately 3 million to one. But H.D. Smyth in his report on the Manhattan project considered what the fission/combustion ratio would be if by fission all the matter of the atom was converted into energy. The equation relating matter and energy $(e = mC^2)$, he wrote,

... shows that one kilogram (2.2 lbs) of matter, if converted entirely into energy, would give 25 billion Kwh of energy. This is equal to the energy that would be generated by the total electric power industry in the United States (as of 1939) running for approximately two months. Compare this fantastic figure with the 85 Kwh of heat energy which may be produced by burning an equal amount of coal.

Smyth knew then, as we know now, that less than 0.1 per cent of the matter in uranium can be converted into energy by fission (to be precise it is 0.091 per cent). His calculation showing a ratio of 3×10^9

to one was therefore knowingly a thousand-fold exaggeration. He also knew what Flügge did not know, namely that only 1 in 139 (usually stated as 1 in 140) of the atoms in uranium is readily fissionable with slow neutrons, that is, those of the isotope ²³⁵U.

As later experience showed, the utilization of uranium by Britain's Magnox reactors is about 0.5 per cent (of which ²³⁵U contributes about 0.35 per cent and the rest comes from the isotopes of plutonium and other elements). Taking a very high grade uranium ore with 1% uranium content, one fifth of which is recovered in extraction, and comparing it with a second-grade, bituminous coal (50% carbon) and some 30% of stone chips and dust our 'from the ground' ratio for Magnox reactors would be:

$$\frac{3 \times 10^6}{100 \times 200} \times \frac{1}{5} \times \frac{100}{35} = 86.1$$

Research carried out in the United States during the Second World War made uranium look much more attractive than the 21,000:1 ratio suggested, for the elements heavier than uranium, elements 93 and 94 in the Periodic Table, were discovered as products of neutron capture by the abundant isotope of uranium, ²³⁸U. By 1941, Glenn Seaborg and co-workers had isolated element 94 and called it plutonium, ²³⁹Pu. As expected from theoretical considerations it proved fissile together with ²⁴⁰Pu and ²⁴¹Pu by both thermal and fast neutrons.

Estimates of world uranium supplies won a new significance in December 1951 when the US experimental breeder reactor (EBR 1) yielded electricity to the grid and it had been shown meanwhile that the number of atoms of the abundant ²³⁸U converted to fissile ²³⁹Pu exceeded the number of ²³⁵U atoms fissioned. That meant that more fuel was 'bred' than was consumed.

The principle of the breeder reactor having been experimentally established. energy forecasters waxed confident about a world energy future based on uranium. Palmer Cosslet Putnam in his report to the US Atomic Energy Commission in 1953 included all low-grade sources of uranium in his calculation of recoverable world reserves at 25 million tonnes, with an energy yield of 1,716Q, one third of which - 575Q — it would be economical to 'burn'. As he was contracted by the commission to estimate the maximum possible contribution which nuclear power could make, it is hardly surprising that this estimate of uranium reserves was wildly exaggerated. Today figures in the range 6.6 to 14.5 million tons have been dismissed as misleadingly fanciful. Total world reserves are still put at less than 2 million tons, plus additional resources of little above 1.5 million tons. When Hans Thirring reworked Putnam's uranium estimates in 1958 he neglected or rejected the one third utilization which Putnam made to allow

for losses during recycling of the fuel in the breeder, with the result that Thirring's value for uranium reserves was 2,000Q. "The date of depletion of the energy capital of our Earth", he concluded in Energy for Man, "is therefore prolonged by at least a millenium . . Thus the consequence of Hahn's great discovery will free us from the fear that our energy resources will run out within quite a short period of history."

US coal resources

A more surprising aspect of Putnam's report was his reworking of the estimates of US coal resources. To the 1944 figure of $3,100\times10^9$ tonnes (total resources) he applied a series of correction factors which reduced it by nine tenths. As Schurr and Netschert have well said Putnam's resulting 3.1Q's worth of recoverable US coal reserves "is based on limiting criteria that have no consistent basis; some of them are wholly arbitrary and some of them have no relation to coal actually in stock".

Nuclear scientists have to their credit that they have in the past cautioned against the acceptance of wildly enthusiastic claims for nuclear power. Such euphoric remarks included the "pill-in-a-pail" suggestion made in the US Congress in 1946. All you needed to heat your house for a year, according to this idea, was a small pill of uranium in a pail of water. Other calculations suggested that a piece of uranium the size of an egg would propel the Oueen Mary from New York to Europe and back, whilst other pundits claimed that one the size of a pea placed under the doorstep would suffice to heat a house for its entire lifetime.

At the end of the Second World War, experience with the Hanford reactors had shown that very little of the 0.7 per cent ²³⁵U in the fuel was 'burnt' before 'poisoning' seriously reduced fission. This led Oppenheimer in 1947 to advise that it did

not appear hopeful to use natural uranium directly as an adequate source of fuel for atomic power. The reactivity of systems based on natural uranium is low; even the fraction of ²³⁵U which can be consumed without replenishing the fuel is small. Because of this the raw material requirements, if such reactors are to play an important part in power economy, are economically prohibitive. It is true that one could re-enrich this natural fuel, but the power expenditure involved in such isotope separation by any present methods makes this likewise prohibitive.

Although the General Advisory Committee to the Atomic Energy Commission discussed drafts of Oppenheimer's memorandum, it was not published for fear of undermining congressional support for the commission. Oppenheimer's ground for pessimism over natural uranium reactors has, as we know, proved exaggerated. Britain's Magnox reactors do 'burn up' some 0.5 per cent of the entire fuel since they fission about half

the ²³⁵U and some of the plutonium they produce. Nevertheless reactors of this type are no longer built, precisely because they use so little of the fuel.

Considerable caution was also expressed at the first nuclear power session of the World Power Conference when the subject was included in this organization's Fuel Economy Conference at The Hague in 1947. Charles Thomas's calculation of the cost of nuclear electricity (nuclear 0.8 cents per Kwh, coal 0.65 cents) for the UN Atomic Energy Commission in 1946 was attacked by Con. Edison's research engineer, Ward F. Davidson and others. Both Sir John Cockcroft and L. Kowarski were cautious, the latter being decidedly caustic about the fast reactor. The tone was again cautious at the full session of the World Power Conference in 1951. Cockcroft said it was "like gazing into the crystal ball to ask us at this stage what operating and capital costs are going to be". At the Atoms for Peace Conference of 1955 he was looking to this "second decade" (the 1970s) for the uranium/coal ratio to be raised by the breeder principle to at least a million to one.

It was in 1951 that work on the fast reactor had begun in the United Kingdom, stimulated by concern over uranium supplies. Eleven years later, the Dounreay fast reactor (DFR) began generating electricity. This 60 MW(t) station was fueled with uranium, 75 per cent enriched ²³⁵U, not plutonium. It was run on full power for five years before being treated as a test bed for plutonium fuels. It has been succeeded by the prototype fast reactor (PFR) using mixed oxide fuel. By 1973 construction was completed and the following year saw operation at low power.

It is surely no accident that the potential world role of nuclear energy was being stressed in the United Kingdom from 1973 onwards based upon the promise of the fast reactor. Its high fuel utilization, claimed Sir John Hill in the magazine Atom, "will enable the vast reserves of very low-grade uranium to be utilized without significantly increasing the overall cost of electricity". World reserves of coal, he admitted, were enormous, but "we are consuming the most accessible and most convenient fuels (gas and oil) fastest and we are depleting most rapidly those reserves which are most conveniently situated geographically, economically and politically". Coal, though abundant, was expensive to mine and transport. Moreover the electricity industry needed flexibility to cope with shortages such as strikes and embargoes could create. Though improvements in UK coal production were impressive, the industry remained in Sir John Hill's words "essentially a labour intensive industry despite increasing use of machinery and, if the men employed are to maintain a standard of living compatible with the task they are being asked to undertake, the labour costs of the industry will rise and this must be reflected in the cost". The

context of these words was provided by the coal price rise of 40 per cent over eighteen months in 1969-70 and the picketing of power stations by mineworkers in 1972. More was to follow — the 140 per cent price rise of coal in 1975, the 400 per cent price rise of oil in 1973-74 and the miners' strike of 1974 which involved a four-month dispute in the industry.

Conclusion

Statistics on fuel reserves do not constitute cold hard facts, they are judgements about the probable outcome of a series of events in space and time. One might even claim tha they do not so much form government policy as result from such policy. Since the introduction of oil-fired power stations in the 1950s the policy of building a multi-fuel base to electricity generation with nuclear base-load has been followed by both political parties. Whereas governments have in the past given some protection to coal against oil there has been from 1955 onwards a consistent policy of favouring nuclear power, a policy shared by the Central Electricity Generating Board whose aim it is to increase dependence upon nuclear-generated electricity for base load, and thus to diminish the impact of higher coal prices and the threat posed by the withdrawal of labour in the coal industry. When the all-party Commons Committee on Energy delivered its report in February 1981, a Daily Telegraph editorial described the recommended "cut-back in nuclear power station construction" as "naive, petty-minded, and ill-informed". The editorial continued:

It is no doubt bad luck for its authors that the report appeared just as an indefinite national coal strike was threatened. But we, on the other hand, should be thankful for the timing. It enables us to see how absurd it would be not to invest massively in nuclear power at a time when coal supplies are under constant threat, and, more important still, when the Russians are reaching out greedy claws towards the Middle East oil wells.

Clearly the formulation of energy policy is a highly political process. The decisions reached can affect the estimates of reserves themselves. Investment in machinery, prospecting, methods of combustion and of fission, all influence what proportion of the total resource may be considered as economically extractable reserves. Thus fluidized-bed-combustion can be achieved profitably with low grade coal of ash content up to 55 per cent instead of the maximum 30 per cent for conventional combustion, thus bringing high-ash coal deposits into the reserves. Similarly highsulphur coals whose combustion causes acid rain can be burnt without liberation of sulphur dioxide in the fluidized bed. Uranium reserves, which at present are trivial by comparison with coal, are increased in terms of their energy yield some sixty-fold with the fast reactor. This, in turn, will allow lower-grade ores to be brought from the resource category and FOR an excellent critical discussion of resource nomenclature the reader is referred to G. B. Fettweiss, World Coal Resources: Methods of Assessment and Resouts (Elsevier, Amsterdam, 1979). Also invaluable are: The Royal Society's submission to the Commission on Energy and the Environment, Environmental Aspects of Increased Coal Usage in the U.K. (February 1980) and the WATT Committee on Energy, Assessment of Energy Resources, Committee Report 9 (1981) and the report of the Commission on Energy and the Environment (1982). The proceedings of the November 1981 British Nuclear Energy Society conference on the fast reactor finel cycle should be published soon. There the "reasonably assured resources" of uranium are given as 2.6 million tonnes.

added to the economically exploitable reserves. There is a sense, then, in which, as Clarke has remarked, an investment decision which significantly favours one energy industry at the expense of the others, is likely to be self-fulfilling, and may thus prove to have been economically and financially correct.

The claimed potentials of both coal and spent uranium fuel reserves raise sensitive issues — fears of increased seismic activity. water table changes and water contamination, in the case of coal mining; concerns about reprocessing and long-term storage, and doubts about the commercial performance of the fast reactor, in the case of spent uranium fuel. If the Coal Board's estimate of 45 × 109 tonnes looks like a 'giant extrapolation' to quote a Nature editorial, how much better are the claims made on behalf of spent uranium fuel? True it has not to be mined, but it will have to be reprocessed many times before even half its energy content has been liberated.

This analysis of energy resource claims does not suggest intentional rivalry by the Atomic Energy Authority and the National Coal Board. Their presentation about the same time was fortuitous. That they both represent responses at the political level in the campaign for government support seems evident. Also clear from this analysis is the fact that the modern distinction between resources and reserves, though dating from 1949, is still in the process of winning general acceptance. Reluctance to adopt it might well be due not only to tradition but also to the tendency which strict definitions of economic reserves have to belittle the long-term potential of an industry's resources. If decision-makers have some appreciation of the factors involved in the process of converting resources into reserves then the application of this fundamental distinction of nomenclature will be more consistently adopted.

I acknowledge the assistance of Mr A. Michael Clarke, Dr Leslie Grainger and Professor E. H. Francis; Mr Keith Bowes kindly commented on an earlier draft of the section of the paper given to nuclear energy. The extract from Oppenheimer's memorandum to the AEC Advisory Committee is cited with the permission of the US Department of Energy.

NEWS AND VIEWS

Not quite full circle? — non-racemic amino acids in the Murchison meteorite

from C.T. Pillinger

In this issue of Nature (p.837), Michael H. Engel and Bartholomew Nagy report finding partially racemized glumatic acid. aspartic acid, proline, leucine and alanine in the Murchison meteorite. They believe the amino acids to be indigenous to the sample, even though full racemization is usually accepted as the indicator that such compounds were present before the meteorite's arrival on Earth. Nevertheless. the authors present a good case and, to their credit, make no attempt to invoke extra-terrestrial biology as an obvious and sensational interpretation of the results. Engel and Nagy only speculate that some type of stereoselective synthetic or decomposition reactions could have taken place on the meteorite's parent body. Less responsible scientists will not be so cautious or await proof that some unusual form of terrestrial contamination was not responsible for the differences between the latest results and samples previously studied.

This publication could be said (unkindly by some) to set back the study of carbonaceous substances in meteorites by some twenty years since it was in 1961 that Nagy, writing with W.G. Meinschein and D.J. Hennessy1, presented the results that sparked off a decade of controversy. Using organic mass spectrometric techniques which were just finding widespread use in the petroleum industry, the group had discovered aliphatic and aromatic hydrocarbons in the Orgeuil meteorite. At the time such precise chemical structures were considered as 'chemical fossils' or 'biological markers' when found in ancient sedimentary rocks and petroleums. The analogy seemed simple - carbonaceous chondrites must be the extra-terrestrial counterparts of such materials. In voicing this conclusion, Nagy and his co-workers were following the suspicions of previous generations who, beginning with Berzelius², questioned whether the results of meteorite analyses were providing "a hint concerning the presence of organic structures in other planetary bodies"

After 1961, a period of vigorous activity ensued, during which many of the classes of compounds common in the Earth's sedimentary regime were located in one or other of a few known carbonaceous chondrites (see ref.3 for a review). Similarly,

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electron microscopic searches for microfossils were initiated and became the subject of acrimonious debate - another controversial area that has just been resurrected (H.D. Pflug, Public Lecture, Cardiff, 26 November 1981).

All the investigations faced the problem that the samples being studied were not fresh; carbonaceous chondrites are extremely porous and friable and some of the most interesting over 100 years old. Who could know what dubious fate had befallen them at the hands of loving collectors and curators? Then, in 1969, the necessary samples were provided. First, on 8 February, a large shower of material landed near Pueblito de Allende, Mexico, almost on the doorstep of the expectant Lunar Receiving Laboratory at Houston. Within possibly hours and certainly two or three days, large samples were being carefully stored free from contamination. Yet despite these precautions, appreciable quantities (1 p.p.m.) of 'biological' hydrocarbon and fatty acids were found4 in exterior, but not interior, portions of the meteorite, presumably having accrued during the brief passage through the atmosphere and sojourn on the ground. The lesson was salutary.

But Allende was still not the right meteorite: that arrived later the same year near Murchison, Victoria, Australia. Some of the first results reported for the sample concerned amino acid analyses5. By this time gas chromatographic techniques had progressed considerably and it was possible to separate amino acid enantiomers, appropriately converted to diastereomeric derivatives, on optically inactive stationary phases. Kvenvolden et al. found that the asymmetric amino acids in Murchison were fully racemized, that is, equimolar mixtures of the possible isomeric forms. Since amino acids from active biological systems are specifically laevo-rotatory or L-form, the authors were able to exclude the possibility that terrestrial organisms had contaminated their sample. Moreover, the distribution of structures was different from that encountered in biological proteins. The fact that simple molecules such as glycine, a-amino butyric acid and α-alanine were most prolific convinced Kvenvolden et al. that they had encountered extraterrestrial amino acids of abiogenic origin.

The Engel and Nagy paper (see p.837) is almost entirely given over to arguments supporting their conclusion that the par-

tially racemized amino acids they find in Murchison are indigenous to the sample. Briefly, these include the observation that the least racemized acids are the most difficult to extract, and the absence or only trace occurrence of common protein amino acids tyrosine, methionine and phenylalanine and the fingerprint indicators serine and threonine. Appropriate controls are offered to demonstrate that the extraction process did not cause the partial racemization or obliterate the evidence of terrestrial contamination. A selected ionmonitoring technique is used to demonstrate that the apparent inbalance in stereoisomer concentrations is not due to some unsuspected co-eluting contaminant. Finally, corroboration of the results was solicited by circulating the samples to colleagues working in the same research area. One less than satisfactory statement is the suggestion that the present study "may not contradict previous reports, because a large interior sample was analysed". Surely small samples would be expected to show inhomogeneities in distribution, not the

Further examinations of amino acids from clean carbonaceous meteorites would indeed be beneficial. What is needed is an absolute indigeneity criterion, independent of stereoisomerism. Not long ago⁶, it was recognized that the total amino acids isolated from Murchison were isotopically very distinct from their terrestrial counterparts and polar extractables from several carbonaceous chondrites are enriched in deuterium7. Perhaps the intramolecular isotopic composition of individual enantiomers will provide the answer; not an easy measurement to perform but feasible in the long term.

Oh that Engel and Nagy had found excesses of p-amino acids, then one could accept the data or suggest some grudgebearing hoaxer had spiked the sample! But that would be even more difficult to explain. After all, we are looking for a source of abiogenically produced L-amino acids to set biochemistry on its discriminatory way.

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Mutualistic interactions among species

from Robert M. May

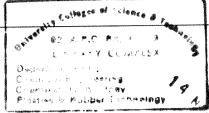
MUTUALISTIC interactions between species have traditionally received significantly less attention from ecologists than have competitive and predator-prey interactions. Recent years have, however, seen growing awareness of this fact, and, in my opinion, empirical and theoretical studies of mutualistic associations are likely to be one of the growth industries of the 1980s. This was one of the themes of the Special Symposium on "Mutualism: New Ecological Theories" held under the auspices of the AAAS at its Annual General Meeting in Washington, DC, in early January.

In opening the Symposium, Boucher observed that mutualism has been cast in the role of the invisible Holy Ghost among the trio of basic types of interaction (see also the review by Boucher et al.1). In elementary biology texts, the contending elaims of competitive and of predatorprey relationships as regulators of population density are usually discussed, while mutualism is dismissed descriptively with some colourful pictures and engaging anecdotes. Most of the current generation of ecology texts compound this disparity: competition and predator-prey typically each have one or more chapters to themselves, replete with discussions of theoretical models and field and laboratory experiments; mutualism is generally treated in a perfunctory way.

As I have suggested elsewhere2, there may be two main reasons for this relative neglect of mutualistic interactions. On the theoretical side, the simple mathematical models developed in the 1920s and 1930s by Lotka, Volterra, Kostitzin and others do capture some of the essential features of competitive and predator-prey dynamics, but they lead to nonsense for mutualism. On the empirical side, the conspicuous mutualistic associations tend to be tropical ones, whereas until relatively recently the bulk of ecological research was done in temperate regions (as a glance at, for example, the cumulative index in the Journal of Animal Ecology3 will show); temperate zone mutualisms certainly exist, but - as we shall see below - they can be more subtle than tropical ones.

Models of mutualistic associations

The non-linear Lotka-Volterra equations describe the interactions between two species in such a way that the per capita birth rate of species A depends linearly on the population densities of species A and of species B. In mathematical terms, this means the 'isoclines', which partition the dynamical behaviour of the populations A and B into various qualitatively distinct regions, are straight lines. For competing species, the results can be victory for species A, or for species B, or



coexistence; more sophisticated models give broadly similar behaviour. For predator-prey, the outcome is a propensity to cyclic oscillations, which again gives the key to the behaviour of more refined models. But for mutualisms that are sufficiently strong, these simple models lead to both populations undergoing unbounded exponential growth, in an orgy of reciprocal benefaction.

As reviewed at the Symposium by Post, a variety of recent analyses have sought to remedy this defect^{2,4-6}. In essence, all these studies observe that the benefit conferred by one species on another will eventually saturate to some limiting value, set by resource or other constraints. The resulting curvilinear isoclines must interesect, giving some finite population level for the mutualists. At the Symposium, Culver⁷ described a specific instance in which the limits to a mutualism (between ants and some spring herbs whose seeds the ants disperse) are apparently set by rodent seedpredators. In many of these models, the mutualistic equilibrium is one where, although both species are more abundant than they would be in the absence of mutualistic effects, both are more dynamically fragile - more prone to fluctuate and taking longer to recover from a given perturbation. On the other hand, as discussed by DeAngelis et al.8, in a patchy environment the mutualistic association between plants and seed-dispersers can act as a stabilizing influence.

Another complication arises when one (or both) of the mutualistic species comes to be 'obligate', in the sense that the population cannot persist in the absence of its partner^{2,4,9}. In this circumstance, there can be two alternative stable states, one with both species present in numbers enhanced by their association and another in which the obligate species is absent (or both are absent if both are obligate). These two alternative states are divided by a 'watershed' of population values, and environmental disturbances can carry the system from one state to the other; in practice, the more likely excursion will be from the state with both species present to the state in which one or both species are absent. An example of such a situation is given by Soberon and Del Rio9, whose model for the association between plants and highly specific pollinators shows that the conditions which optimize the production of nectar tend to diminish the stability of the association.

Elaborating a general notion introduced by Levine¹⁰, Vandermeer described how

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effectively mutualistic interactions can arise indirectly in complex food webs11. Suppose species A and B appear to compete for prey species C and D, with species B concentrating mainly on resource D. Suppose further that species C is competitively superior to D and would eliminate D were it not for the effects of the predatory species A. It now can happen that removal of species A will lead to the prey species C displacing D, thus decreasing the population of B which is sustained largely by D. Thus the apparently competing species A and B are, in fact, mutualistically related, by virtue of these 'indirect' interactions. As Vandermeer showed, when realistic time lags are added on top of these indirect effects, the dynamics of mutualistic associations can become very complex indeed. There is clearly much room for further theoretical

Empirical studies of mutualism

Many beautiful studies have recently been carried out, analysing the energetic costs and benefits involved in the association between nectar-producing plants and their pollinators. The plant-pollinator systems are mainly tropical, and often obligate¹²; the pollinators embrace butterflies^{13,14}, hummingbirds and sunbirds¹⁵⁻¹⁷, bats^{18,19}, bees^{20,21}, lemurs and marsupials²². Likewise, recent work on some tropical associations between plants and seed-dispersers has not only analysed costs and benefits, but has shown the longterm dangers inherent in obligate mutualisms. Thus, unless we intervene, the Calvaria tree looks like following to extinction the dodo that once dispersed its seeds23. More generally, the neotropical flora may still be changing in response to the disappearance of many large mammals from South America²⁴. Fascinating though these studies are, here we will concentrate on mutualisms involving plants, anttended insects and ants.

The tropics afford many striking examples of obligate ant-plant associations, in which plants provide both food and housing (in special 'apartment-like' physical structures at the base of thorns or elsewhere) for ants. In temperate regions, associations in which plants offer food (but not housing) exist, albeit less conspicuously.

Inouye and Taylor²⁵, for example, have shown that Aspen sunflowers possess extrafloral nectaries that attract ants. By excluding ants from some plants, and by other manipulative experiments in the field, these authors have demonstrated that the ants confer reciprocal benefits on their host plants, by providing a degree of protection against herbivorous predators. In a similar set of carefully designed field

experiments, Tilman²⁶ has shown that the extra-floral nectaries on black cherry trees attract a species of ant, which in turn helps check the depredations of tent caterpillars. A variation on this theme is provided by Messina's work²⁷ on the ant species attracted to goldenrod by the nitrogen-rich 'honeydew' they can get from tending membracids (tree-hoppers) that are feeding on the plant; as observed by Bristow²⁸, in such circumstances the phytophagous insects are a kind of portable extra-floral nectary. Here Messina's manipulative experiments again show that the ants help to exclude other herbivores from the plant.

The above studies carefully document the costs and benefits of the ant-plant association. On the other hand, they typically do not wrestle with the complexities of food web structure, often lumping different ant species together as 'ants'. The complications that can arise in unravelling the full community structure of an ant-homopteran-plant association is well illustrated by the pioneering work of Addicott²⁹: at his study site, fireweed supported four species of aphids, of which three were tended by a total of ten species of ants. This detailed investigation of community structure is, however, largely descriptive.

Of particular interest, therefore, are some recent studies which combine manipulative and analytical techniques with full dissection of the structure of the community. One such study, involving carrion-burying beetles and hitchhiking mites, was described at the Symposium by D.S. Wilson; a full report of this complex story will be given another time. Another such study involves — in its essentials — a

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membracid and an aphid species that feed on ironweed in New Jersey. These homopterans are tended by two ant species, a Myrmica and a Tapinoma. Using controlled experiments in the field and in a greenhouse, Bristow²⁸ has elucidated the detailed structure of this community, along with the costs and the benefits of the association to both ants and homopterans. Under favourable conditions, there is a strong tendency towards specialization by the ant species: Myrmica prefers membracids, and Tapinoma prefers aphids. This tendency is, however, far short of an obligate dependence: the preferences are not exclusive, and either species of ant will tend either species of homopteran. Bristow²⁸ goes on to observe that in temperate zone systems, absolute dependence is often too costly. Winter forces a prolonged break in any association, in time and perhaps also in space; species exploiting ephemeral hosts must

find new hosts at least once each spring, and possibly more often. Ants cannot rely on their highly localized foraging area being recolonized by their preferred honeydew producer. By the same token, homopterans may find themselves abandoned if the ants move for some reason.

Bristow²⁸ concludes by suggesting a new paradigm for mutualistic associations outside the tropics:

"The interesting point is not that temperate zone mutualisms are rarely obligate, but rather that by relying on simple environmental cues and behavioural mechanisms a high degree of fine tuning may be achieved with almost no loss of flexibility. This suggests that facultative mutualisms may deserve a closer look than they have received; their contribution to community or guild structure may not be as negligible as has been previously presumed."

Type I supernova thermonuclear versus collapse

from Stirling A. Colgate and Albert G. Petschek

In a recent issue of Nature, Canal, Isern and Labay1 discussed the rather esoteric problem of the evolution of a type I presupernova core - a white dwarf star where a eutectic phase separation of carbon and oxygen is presumed to take place before explosion. This would seem to be a singularly esoteric and even abstruse issue. but such are the labyrinthian relationships in science that this topic is central to a major issue in astrophysics - the mechanism of supernovae. It depends in turn on the most mundane property of metals - the alchemy of alloys and the long-range order functions of the fluidsolid transition, a controversial topic in theoretical physics. The same problem arises in the analysis of ordinary materials as well as of y bursts.

A supernova — the explosion of a star is the most dramatic real-time event that we observe in the Universe. Presumably, most of the elements necessary for life are generated and dispersed within the Galaxy by such an event. Presumably also, the most exotic energy and state of matter. cosmic rays and neutron stars, are formed in a proportion of such events. Yet there is no universal agreement as to the mechanism(s). The large star, \sim 10 solar masses (M_{\odot}) , almost certainly makes a type II supernova, leaving a neutron star remnant. The collapse and subsequent explosion is currently modelled by the elastic bounce energy of the forming neutron star core^{2,3}. In the past, neutrino emission was thought to cause the explosion that accompanies collapse, but now neutrinos are believed to hinder the

propagation of a mass-ejecting shock. The theoretical outcome, that is, explosion or collapse to a black hole, is still dependent upon details. The need to resolve this unhappy uncertainty has stimulated considerations of possible different neutrinomatter interactions, such as axions4 and Majorana coupling⁵. Violent neutron star convection has also been considered by Colgate and Petschek6, and refuted by Lattimer and Mazurek7.

Type I supernovae are believed to be older, less massive stars that ignite a thermonuclear reaction in the core and are pushed over the edge of degeneracy stability - the Chandrasekhar limit - by a combination of slow accretion from a binary and evolution. If a carbon core ignites early at the low density of $2-5 \times 10^9$ g cm⁻³, then the star explodes from a thermonuclear deflagration and no remnant is left. However, if an oxygen core thermonuclearly ignites at the higher density of $\sim 2 \times 10^{10}$ g cm⁻³, then rapid electron capture decreases the degeneracy pressure faster than thermonuclear deflagration drives expansion, and collapse to a neutron star occurs8. We are then left with the same difficulty - how does a type II supernova work? Nevertheless, we can predict that a carbon deflagration disintegrates the star and ejects all the matter (1.41 M_☉), mostly as iron, into the Galaxy. An oxygen deflagration and collapse would eject

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about one-fifth as much. This latter case is more comfortable for galactic theorists but comfort is not everything9. The light from the supernova comes from the subsequent ß decay energy of 56 Ni that decays to stable iron (56Ni 6.6 day 56Co77 day 56Fe). The classic observed optical decay half life of 56 days of supernovae I10 is powered by the radioactive decay positrons from ⁵⁶Co. An increasing proportion of the β^+ can escape and simulate a 56-day exponential provided the ejected mass is relatively small. The larger ejected mass resulting from carbon deflagration models requires a critically timed IR emission11 if agreement with observation is to be obtained. The positrons, which were once observed at the galatic centre and now seem to have disappeared^{12,13}, would have had the correct flux if the source were a supernova type I involving collapse with the escape of 10 per cent of the positrons.

Helium burning in the pre-supernova white dwarf star leads to roughly a 50:50 mixture of fluid carbon and oxygen at a density of 108-109 g cm⁻³. A eutectic exists in the phase diagram (66.8 per cent 12C and 33.2 per cent 16O), as in a lead-tin solder. On energetic grounds, the smaller atom or ion prefers to fill the interstices of the lattice of the larger one and this lowers the melting point. The excess component, 16O, precipitates out as 16O snowflakes or perhaps as glass-like spherules and falls to the centre of the star. A further separation may occur after remixing of the remaining fluid due to a gravitationally induced thermohaline instability. Depending on the degree of separation of the core, an oxygen-rich core in the white dwarf will lead to a collapse, not a thermonuclear disintegration.

The energetics of the liquid-solid transition of matter is modelled in extensive Monte Carlo calculations that lead to melting point predictions¹⁴. Crystallization, on the other hand, requires additional physics for the transition to take place and has not yet been modelled numerically. The possibility of a neutron star quake leading to a neutron star volcano and subsequently a y burst depends on the same physics of the melting transition of neutron star crust matter¹⁵.

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Struggles among the dunes

from Peter D. Moore

THE manner in which one plant paves the way for another in the course of vegetation succession may sometimes have its ironic aspects. So often, a colonizing plant species nullifies the effect of a severe environment only to find that, in doing so, it has made possible an invasion by a competitor that brings about its own local extinction.

Take, for example, a habitat which is close to the hearts of all students of succession — the sand dune. In West European dunes, the grass Agropyron junceiforme is the most conspicuous primary colonist of sands around high-water mark, being tolerant of high salinity (up to 6 per cent according to Salisbury1) and prolonged immersion. As it grows it creates wind eddies and encourages local sand deposition, and so raises minor dunes around itself. The sand surface rises further and further above the sea, until Ammophila arenaria, the marram grass, which has a lower salinity tolerance (about 2 per cent), is able to invade. The higher stature and aggressive, tussocky growth of the marram grass leads rapidly to its dominance. The subsequent decline of Agropyron seems simply to reflect its failure in the struggle for survival under competitive conditions.

At this stage many annual plant species invade and much consideration has been given to the factors which limit their establishment ¹⁻³. The young dune-front is exposed to adverse conditions but they become less severe as succession proceeds; salt-spray, shifting and unstable sands, low water availability and high diurnal temperature amplitude all may play their part.

Van der Valk4, for example, found the seedlings of invading forbs to be more sensitive to sand accretion than to any of the other above factors. In Cape Hatteras, North Carolina, 20-30 cm of winter sand is deposited on the coastal foredunes. But the seedlings can cope with only 5 cm of sand and their seeds with burial by 1-16 cm, so forbs can invade only when the major dominants, such as Ammophila breviligulata, have stabilized ridges and produced sheltered locations within the developing undulations and hollows. In the same way, A. breviligulata's European counterpart, A. arenaria, produces stabilized dune systems in the European coastal fringe, which support a high diversity of adventive species⁵.

What is very much less clear, because of the difficulty encountered in subjecting any given hypothesis to experimental testing, is why the major dominants like Agropyron and Ammophila become less

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vigorous, flower less frequently and finally die out as the succession proceeds. The obvious answer is that subsequent species have greater competitive ability. But this does not always hold true, for the demise of Ammophila in older, stable dunes may not be accompanied by aggressive dominance of the system by robust newcomers. Moribund marram is often surrounded by a short turf vegetation rich in lichens and mosses which do not seem to offer a serious competitive threat; sometimes the declining Ammophila populations are accompanied by very little vegetation at all⁶.

Obviously other factors than competition are at work. Salisbury1 considered increased soil compaction and acidity as possible influences but it was Marshall7, whilst working on another sand dune grass. Corynephorus canescens, who hit upon a rather surprising answer. He found that burial of Corynephorus tufts encouraged the growth of new adventitious roots from tillers of grass and caused its tussocks to spread and proliferate. It grew well under conditions where sand accretion amounted to 10 cm per annum, but became moribund on stable sand and failed to generate new roots. The direct effect of burial was to increase the humidity around the tillers necessary for healthy root growth. Hope-Simpson and Jefferies⁶ confirmed many of the points made by Marshall concerning root growth for Ammophila, except for one piece of evidence. When planted in a Bristol garden Ammophila grew luxuriantly, both in foliage and root, and flowered freely despite the stability of the

Recently, interest has developed in another dominant plant of dune successions, the sea buckthorn, Hippophaë rhamnoides. It is undoubtedly a native plant in Britain8, but its frequency in coastal dunes, especially in the west, is probably a consequence of deliberate planting to stabilize sand9. It is a shrub which lives for about 40 years 10 and has root nodules in whch symbiotic nitrogen fixation occurs11. Its growth can, therefore, have considerable ecological consequences and raise ecosystem nitrogen levels by up to 179 kg ha-1yr-1 in sand dunes¹². Stewart and Pearson, who conducted studies on the nitrogen-fixing capacity of Hippophaë, noted that nodules on the youngest plants were most active in fixation.

On dunes in the Netherlands, Hippophaë enters a degenerate phase in older dunes, in a similar way to Ammophila. Oremus and Otten¹³ grew vigorous, young Hippophaë in soils from young embryo dunes and from stable sites where the Hippophaë was in a post-optimal state of development. More root nodules

were produced in the embryo dune soil and there was a higher rate of nodule death and necrosis in the mature dune soil. Root growth generally was poorer in the mature soil, roots were darker and root hairs fewer. Gamma irradiation of the soil samples before planting removed these root effects and healthy root systems developed on the plants even when grown in mature dune soils, which suggests that an organism within the soil was responsible for root stunting. A possible culprit was considered — the nematode Longidorus. It is absent in the embryo dune soil, but common in that of the mature dune and belongs to a genus known to cause root damage among many plants.

Several possible developments suggest themselves from this work. First, this knowledge of the susceptibility of Hippophaë to Longidorus infection, if confirmed, could provide a technique for the biological control of Hippophaë in those sand dune nature reserves where sea buckthorn has proved a pest by forming such dense and invasive patches that overall species diversity has been

reduced10. Second, the role of soil fauna in other cases where vigour declines as soils mature, such as Ammophila, needs to be investigated. It could account for the healthy growth of marram in the stable gardens of Bristol. Third, the work should serve to underline the potential importance of the soil biota, both faunal and microbial, in influencing competitive interactions and succession in the field.

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Somatic gene amplification during Drosophila oogenesis

from David Ish-Horowicz

THE construction of an egg can make large demands on macromolecular synthesis. especially for organisms in which oogenesis is particularly rapid. Such is the case for Drosophila melanogaster, in which oogenesis takes only 2.5 days and as many as two to three eggs an hour can be laid. The intense synthesis of oocyte components is mainly achieved in cells whose DNA content has been amplified up to 1,000-fold by an increase in chromatid number per chromosome (polytenization). Two recent papers 1,2 show that, in addition to a 45-fold polytenization of the chromosomes in the follicle cells producing the eggshell, there is a further novel mechanism to boost synthesis: a localized differential polytenization of the gene clusters coding for eggshell components.

The protective eggshell, or chorion, contains about 6 major and 14 minor proteins that are synthesized over about 5 hours in a characteristic order that reflects the shell's complex multilayered structure3,4. Two years ago Spradling and Mahowald⁵ showed that the chorion genes are selectively amplified in follicle cell nuclei but not in other tissues. Using cloned cDNA and genomic DNA fragments two clusters of chorion genes were studied: one mapped to the X chromosome within

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bands 7F1,2 and includes the genes for proteins s36-1 and s38-1 and two related but uncharacterized genes⁵⁻⁷; the other cluster derives from the third chromosome (between bands 66D11 and -15) and encodes proteins s15-1 and s18-1 and includes two other genes 7.8. Each cluster is expressed for only 1-2 hours and appears to be coordinately regulated^{4,6}.

Comparison of egg chamber DNA with that from other male and female tissues showed5 that, in addition to the polytenization, the s36/s38 cluster is amplified about 12-fold, and the s15/s18 cluster is amplified about 60-fold. Differential replication is maximal at stages 11-13, the time of chorion protein transcription. At typical rates of transcription, about a 10-fold amplification would be needed to make the observed levels of s36 mRNA in 2 hours5, suggesting the extra genes are necessary to make sufficient chorion proteins.

Two possible models of amplification were suggested: additional extrachromosomal chorion genes, or localized differential polytenization. The two recent papers strongly support the latter explanation.

Spradling1 examined the regions surrounding each cluster and showed that the amplification extends well beyond the chorion genes. Even as far as 40 kilobases (kb) away from the cluster, he finds two-to fourfold amplification. Maximal amplification occurs at the chorion genes, with a steady decline in more distant regions, consistent with a replication origin mapping close to the cluster. The steady decrease in amplification suggests the replication terminates randomly rather than at occasional discrete sites. He confirmed the absence of specific termination sites by using Southern transfer hybridization to look for restriction fragments with an altered electrophoretic mobility. No such differences between amplified and nonamplified DNA were found.

Elegant confirmation of the model is presented in the second paper2 where Spradling and Mahowald studied a femalesterile mutation occelliless (oc)9 caused by a 10-band chromosomal inversion which breaks the s36/s38 cluster, transposing most of it next to sequences in 8A1,2. The mutation drastically alters the pattern of amplification, as if the inversion has transposed a cis-acting replication origin. The inverted chorion genes are still amplified and the replication spreads into the adjacent and previously unamplified 8A1,2 sequences. In contrast, the uninverted 7F1,2 sequences (including at least one chorion gene) are no longer amplified. The replication origin must thus be in the proximal part of the cluster and, indeed, this is the first strong evidence for a discrete eukaryotic chromosomal replication origin. As well as disrupting the pattern of amplification, the oc inversion reduces s36 and s38 amplification twofold by inhibition of the last round of somatic amplification. The consequent reduction of s36 and s38 synthesis may contribute to the female sterility (which is due to defective eggshell production¹⁰), but other reasons may include the inability to amplify the untransposed chorion gene.

Is tissue-specific gene amplification during development a common mechanism for selectively enhancing gene expression? This may be the case in Drosophila where many, if not most, differentiated tissues

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are polytene. A long-standing example comes from the salivary glands of Rhynchosciara where DNA amplification of a chromosome band (DNA puffing) precedes its transcription¹¹. In this case, the trigger for DNA replication is the steroid hormone ecdysone¹². Perhaps chorion gene amplification in Drosophila is also under hormonal control although there is only suggestive evidence for this. Both ecdysone and juvenile hormone have been implicated in regulating vitellogenesis in Drosophila^{13,14}, which occurs at the same stages as the chorion gene replication. Drosophila mutants that are temperature sensitive for larval ecdysone production are also temperature sensitive for female fertility and eggshell production 15,16 but the latter effect appears associated with follicle cell degeneration¹⁷. As chorion maturation has been demonstrated in cultured ovaries¹⁸, the role of hormones in triggering somatic chorion gene replication is now accessible to *in vitro* study.

It is doubtful that tissue-specific gene amplification is very general. The chorion of the silkmoth *Bombyx mori* is also secreted by surrounding follicular cells, but its proteins are made from over 100 linked genes^{19,20} that are not thought to be somatically amplified. Indeed, a host of other intensely transcribed genes are single copy in expressing cells. Presumably, the rarity of somatic gene amplification reflects the ability of a single protein-coding gene to cope with heavy synthetic requirements, except under the most demanding circumstances.

Some like it hot: temperature-determined sex

from Paul H. Harvey and Montgomery Slatkin

Most biologists are familiar with chromosomal sex determination. Mammals with both an X and a Y chromosome develop as males while those with two X chromosomes develop as females. In birds the arrangement is reversed and females are the heterogametic sex. Some reptiles, amphibians and fishes also possess sex chromosomes which determine whether the zygote develops as a male or as a female!

Genetically controlled determination has generally been assumed to have a remote ancestry in the vertebrate lineage, but recently a quite different sexdetermining mechanism has been discovered. Sex in many reptiles is now known to be not genetically controlled but determined by the incubation temperature of the zygote during embryogenesis².

The first case of temperaturedetermined sex was recognized in 1966 for a lizard3, and others appeared among turtles in the 1970s4,5, but it was not until 1979 that Bull and Vogt6 ruled out the possibility that differential mortality dependent upon temperature was the source of the sex-ratio bias. In their study of five species of turtle, they were further able to show that, in four of the species, sex ratios in the wild resulted from the degree of exposure of the nest to sunlight and hence to the incubation temperatures. In the fifth species, sex determination was not temperature dependent. Among reptiles, temperaturedependent sex determination is now known or suspected in alligators, two species of lizards and many turtles2 (including sea turtles7 - with important implications for conservation measure for endangered species). Most reptiles have yet to be studied.

Incubation temperatures can have a despecie offset on severatio bias. In many

incubation of eggs in the laboratory as a

dramatic effect on sex-ratio bias. In many turtles, low temperatures (20-27°C) produce only males, high temperatures (30-35°C) produce only females and there is a narrow temperature range producing both sexes. Lizards show the opposite pattern with low temperatures causing eggs to develop as females, and high temperatures as males. Snapping turtles (Chelydra serpentina) show yet a different pattern of response: females develop at high and low temperatures (20 and 30°C) and males in between. But natural nest sites fluctuate in temperature. In a laboratory experiment subjecting map turtle (Graptemys) eggs to daily temperature fluctuations between different all-male and all-female extremes (20-30°C and 23-33°C)6, the broods raised between the lower temperatures produced only males, and those between the higher temperatures

In species which have heteromorphic sex chromosomes (those recognizable under a microscope), it seems that the environment does not also determine sex, that is, individuals which deviate from the usual association of XX with one sex and XY with the other are not observed (for a review, see ref. 2). There may, however, be a genetic component to temperaturedetermined sex. In the Atlantic silverside (Menidia menidia)8, low temperatures produce more females but eggs from the broods of different females show different temperature-response curves. Similar, though less extreme effects have been noted in turtles9.

Little is yet known about how tempera-

ture controls sex determination. The sensitive period lasts some weeks during the middle of development in turtles 10-12 and occurs about 46 days after hatching in the Atlantic silverside. Temperature may thus be acting during gonadogenesis. H-Y antigen, a cell-surface component suspected of having a primary role in gonadal sex determination in vertebrates (the heterogametic sex is H-Y positive)13, has been studied in fourteen turtle species14. In thirteen species the female was typed as H-Y positive, and in the other species (Chinemys reevesi) the male was H-Y positive. Unfortunately, very few individuals of each sex were typed (between one and three) and so the data do not provide firm evidence for a completely sexdifferentiated response. Indeed, in two species the male was not typed and a previous study had shown that, in a third species (Emys orbicularis), both males and females could be either H-Y positive or H-Y negative¹⁵. This species shows temperature-dependent sex determination, so the results suggest that H-Y antigen does not control sex differentiation in this species. If the findings are correct and eventually hold for gonadal cells (the quoted experiment was performed on blood cells), this constitutes a major departure from the supposed ubiquitous role of H-Y antigen in vertebrate sex determination.

Temperature-dependent sex determination poses both demographic and evolutionary puzzles. What, if anything, prevents large variations in sex ratio in both space and time as developing broods respond to different climatic conditions? Bull and Vogt16 compared sex-determining temperatures among embryos of map and painted turtles collected from the northern and southern United States and predicted that embryos from southern populations should develop as males at higher temperatures than those of northern populations. Interspecies comparisons provided no support for, and intraspecific comparisons (on two species where this is possible) actually went against, the hypothesis. If this is generally true, then there is no local adjustment of the temperature-dependent mechanism through zygotic means. Populations will then exhibit large geographical differences in sex ratio unless some compensatory mechanism, such as varying the choice of nest sites or emigration, adjusts the adult sex ratio.

Why has temperature-dependent sex determination evolved? If environmental conditions cause a large excess of one sex, then, following Fisher¹⁷, any mechanisms that reduced the excess would be favoured by natural selection. Charnov and Bull¹⁸ have argued that environmental sex determination (of which temperature-determined sex is one case) would evolve when conditions experienced during some stage of development have a strong differential influence on the fitnesses of

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adult males and females. For example, larval mermithid nematodes become female in response to high levels of nutrition (in large hosts), but become male under low levels of nutrition. The explanation is that the large individuals, that can develop in large hosts, can produce more offspring as females than as males. At present, it is not clear whether such effects exist in reptiles but a new study of alligators (see this issue of Nature, p.850) suggests that they may. Incubation temperature indirectly affects the animals' growth rate by influencing the amount of yolk remaining at hatching. Large size early in life is apparently more beneficial to females than to males.

Temperature-determined sex may indeed be the ancestral condition for reptiles. It is known in three of the four living orders: turtles, snakes and lizards, and crocodilians (the tuatara, the surviving representative of the Rhyncocephalia, has not been studied). Morphologically distinguishable sex chromosomes are found in only two orders (the turtles and the snakes and lizards), but it is uncertain

whether genotypic sex determination originated once or more than once². If temperature-determined sex is ancestral for reptiles, and if the birds and mammals arose from such a reptilian stock, then we are left with a radically different view of the evolution of sex determination in the vertebrates.

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An Arctic Ocean ice sheet in the Pleistocene?

from L.D. Keigwin

An intriguing and controversial suggestion stemming from recent palaeoclimatic studies is that the Arctic Ocean was covered by a massive floating ice shelf during the last glacial maximum. The idea has been put forward from evidence of 618O fluctuations in deep-sea cores and in ice cores on Greenland1 and has been proposed as a solution to problems in Arctic glacial dynamics and chronology².

A recent paper by Williams, Moore and Fillon³ now puts the floating Arctic Ocean ice shelf hypothesis in a timestratigraphical framework for the entire Pleistocene, and for the last 150,000 years in particular, by comparing inferred sea levels from the δ^{18} O records of the deep sea with the record of sea level given by raised coral terraces. They found significant differences in the apparent sea level calculated from the two data sources which they attributed to the presence of a floating ice shelf that would produce the change in seawater isotopic composition without significantly altering sea levels. The authors conclude that ice shelves may have originated as important features earlier in the Quaternary.

Oceanic 818O records have been observed to change from high-frequency, low-amplitude to low-frequency, highamplitude variability about 900,000 years before present. Williams et al. argue that at that time the Earth's orbital parameters

changed and allowed the first combined accumulation of massive ice shelves and sheets in the Arctic.

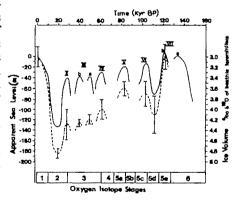
For the Quaternary it is widely held that δ18O changes of 0.10°/00 in deep-sea benthic foraminifera correspond to sealevel changes of 10 m (based on a mass balance assuming average δ^{18} O values of glacial ice4). A similar relationship has been derived from 618O variations in corals from uplifted terraces⁵. The only known way rapidly and greatly to enrich the ocean in ¹⁸O is by removing isotopically lighter ¹⁶O from the ocean through evaporation and precipitation. A fall in sea level will result if the precipitation is stored on continents as ice, but not if it is stored as a floating ice shelf. The 518O of deep-sea benthic foraminiferal calcite depends on the temperature and isotopic composition of the seawater and so-called 'vital effects', but most of the 1.7% o glacial-interglacial δ¹⁸O change in the ocean can be attributed to seawater compositional changes caused by increased ice volume. Vital effects can be reduced by analysing single species of benthic foraminifera and the effect of changing deep-sea temperatures on 618O records is generally discounted because the total amount of isotopic change would

L.D. Keigwin is at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts represent a cooling of most of the deep sea below freezing.

Sea-level records based on raised coral terraces are not continuous, but can be dated. Constant rates of uplift have been assumed and, where reasonable, a composite sea-level record has been derived. Williams et al. compared such a composite terrace record with a composite deep-sea record for the late Ouaternary3 to arrive at their conclusions. Other sea-level indicators, however, do not always agree with the raised coral terrace record.

Hollin6 has provided evidence for a dramatic rise in sea level about 95,000 years ago which may be due to an Antarctic glacial surge. If such events occur in less than the 1,000 year mixing time of the ocean they may not be resolved in the deepsea stable isotopic record. The greatest apparent sea-level differences between the generalized records of Williams et al.3 are between about 20,000 and 100,000 years ago where they differ by 30-80 m. To produce a 50 m sea-level difference the area of the Arctic Ocean would have to be covered by a floating ice shelf 1,300 m thick, close to the mean depth of that ocean. Even if adjacent seas and part of the North Atlantic are included, hundreds of metres of ice shelf are still required.

Alternatively, the 50 m sea-level difference (equal to a difference of $\sim 0.50\%$ could be accounted for by a deep-ocean cooling of 2°C. Williams et al. have not given this possibility emphasis because of ambiguities in inferring bottomwater mass history from benthic foraminiferal distributions. Studies of cores under Antarctic bottom water show faunal changes that do not follow a simple glacialinterglacial pattern^{7,8}, but studies of cores beneath deep waters (Circumpolar and



Comparison of apparent sea-level records from raised coral terraces (solid line) and benthic foraminiferal oxygen isotope records (dashed line). The isotopic record is a composite of results from five Atlantic, Indian and Pacific Ocean deep-sea cores and error bars represent one standard deviation about the mean at various times. Vertical scales are adjusted so that a 1.1% of 18O change equals a 10 m sealevel change. Sea-level differences between the two records of as much as 80 m may result from ice shelves which could change the isotopic composition of the ocean without significantly lowering sea level, and from cooler deep-sea temperatures during glacial intervals.

North Atlantic deep water) do show glacial-interglacial faunal oscillations, suggesting water mass changes 9,10. The isotopic data examined by Williams et al. are based on sediment from cores of relatively shallow water depth (< 3,300 m) within deep-water masses. Deeper cores generally have lower sedimentation rates because calcite dissolution increases with depth; thus they have lower resolution. As such, most cores from which isotopic data have been reported actually could have 2°C glacial-interglacial changes in deep-water temperature.

Deep-sea cooling could be eliminated from consideration by studying stable isotopes in cores where deep-sea water is less than 2°C from freezing. Water mass changes could also significantly affect the interpretation of benthic foraminiferal isotopic records as ice volume-sea level indicators because the range of the principal modern deep-water masses is about 0.6%. For example, a seawater compositional change of this size could result from replacing North Atlantic deep water by Antarctic bottom water at the transition from an interglacial to a glacial interval. Recent stable isotopic and geochemical studies of cores within the Atlantic suggest reduction or elimination of North Atlantic deep water during glacial intervals11-14.

Regardless of the uncertainties in interpreting foraminiferal 818O data, some glaciologists have hypothesized that during glacial extremes there were massive ice shelves associated with an Arctic ice sheet^{2,15,16}. This controversial hypothesis is based on analogy with the Antarctic ice sheet, a large part of which is currently grounded below sea level. Central to the idea2 is the role of ice shelves in explaining the location, mode and sequence of formation of most Northern Hemisphere ice domes. Briefly, Hughes et al.2 propose that massive ice shelves form when ice streams coalesce and the shelves buttress and allow the growth of massive domes. The shelves must form before the domes, so the largest ice volume growth in the late Quaternary would require ice shelves.

The work of Williams et al. has focused attention on the debate about the extent of floating Arctic ice during glacial intervals. Although evidence from Arctic Ocean cores indicates perennial sea-ice cover began 700,000 to 900,000 years ago17, it is difficult enough to prove the existence of massive ice shelves during the last glaciation, not to mention earlier in the Ouaternary. Further studies of deep-sea cores, including cores from the Arctic may help resolve the problem.

The key to the schistosome's success

from A.J.G. Simpson and D. Cioli

SCHISTOSOMIASIS or bilharzia is a disease affecting some 200 million people in the developing countries. The causal agent is a parasitic worm, the schistosome, which lives in the blood vessels of the intestine or urinary tract; man becomes infected by the cercarial, or free-living aquatic, stage which penetrates the skin directly. The key to understanding how host immunity is effected and how the worm evades this response is to learn more about the schistosome's double outer membrane (DOM). This unique surface not only mediates crucial metabolic functions but also counteracts the immune system of the host. Progress in this field was reported at a recent meeting in Geneva*.

The techniques of subcellular fractionation are providing an invaluable approach to the characterization of the DOM. It can be selectively released from the parasite simply by brief incubation in buffered saline at 37°C (Evans, NIMR, London), by using freeze-fracture techniques (Wilson, University of York), or by utilizing the negative surface charge of the parasite as the basis of membrane purification (Cesari, Instituto Venezolano de Investigaciones, Caracas). An alkaline phosphatase of broad specificity has been widely used as a marker for the DOM and enrichment of specific activity for this enzyme has been reported, by all investigators, in fractions shown to contain DOM by morphological observation or in those enriched in externally applied ligands.

Wilson and Podesta (University of Western Ontario) have further dissected the DOM by preparing separate fractions enriched for the inner and outer bilayers. Podesta utilized the sequential exposure of the parasite to 0.1 per cent digitonin solutions to obtain first the outer and then the inner bilayer from intact adult worms, a technique previously used in the preparation of mitochondrial membranes in other systems. The outer bilayer fractions had very low enzyme activities, suggesting that membrane-bound enzymes are associated predominantly with the inner layer of the DOM.

The surface of the schistosome represents the primary target for immune attack. As the schistosomulum (the immature worm) is the most susceptible stage, attempts to determine target antigens have largely been conducted by surface labelling this stage. Several participants reported results carried out by a variety of techniques. As many as ten or more proteins of molecular weight 18,000-200,000 may be labelled on schistosomula. There is, however. variation in the polypeptides labelled by the various procedures, and Cioli (Consiglio Nazionale della Richerche, Rome) has shown that the most selective and gentle methods identify an 18,000 molecular weight polypeptide. Not all polypeptides can labelled immunoprecipitated with sera from patients or infected animals and the reported molecular weights of major precipitated antigens vary considerably.

Taylor (University of Cambridge) used iodosulphanilic acid to label the schistosomulum and immunoprecipitated polypeptides of molecular weights 185,000, 105,000, 68,000 and 24,000 with sera from infected humans. Dissous (Institut Pasteur, Lille) immunoprecipitated polypeptides of 40,000, 37,000 and 32,000 with immune rat sera following lactoperoxidase-catalysed iodination of the schistosomular surface. Both of these investigators reported the production of monoclonal antibodies which recognized defined surface antigens (Taylor molecular weight 24,000, Dissous -37,000) and detected some in vitro activity with their monoclonal reagents. Dissous further reported the passive transfer of immunity to rats using her monoclonal antibody.

How is the DOM of the developing parasite involved in immune evasion? Susceptibility to immunity is age dependent and as the parasite matures it quickly loses the ability to bind antiparasite antibody, a change which correlates with the detection of host deter-

*This meeting was organized by the Scientific Working Group

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minants on the parasite surface. Caulfield (Harvard Medical School) reported, however, that parasite antigens are spontaneously lost from young schistosomula in vitro, even in the absence of serum or other macromolecules. Thus, the loss of antigenicity may result from the active shedding of parasite surface components.

Membrane turnover and shedding (particularly in response to ligand and cell binding) may be generally involved in the protection of the parasites. Brink (Harvard Medical School) demonstrated the shedding of antibody-antigen complexes from in vitro cultured schistosomula, and Caulfield reported that following a unique membrane fusion between the schistosomulum and neutrophil membranes, whole areas of membrane may be lost to the cell interior, but the parasite survives and eventually the neutrophils are released. Kemp (Texas A & M University) has also detected immunoglobulins bound to the adult surface via their Fc portion, and if anti-Fab antibodies are bound to the surface immunoglobulins, the complexes are actively shed within 15 minutes. Freezefracture (Torpier, Institute Pasteur, Lille) shows that this is accompanied by the cross-linking of membrane components (visualized as intramembranous particles) into tetrads and their subsequent disappearance from the DOM.

Alterations in the chemical composition of the double outer membrane may result in its being refractory to the effects of cytotoxic mechanisms. When five-day schistosomula are recovered from mice

(McLaren, NIMR, London) they are coated with red blood cell antigens. If the parasite is exposed to eosinophils in the presence of anti-mouse red blood cell antibody, the cells bind to the surface and degranulate, but fail to kill the parasite although similar antibody-dependent cytotoxic mechanisms are potent killers of fresh schistosomula, and also of parasites three weeks old or more. Thus, even if the surface components of five-day old worms are recognized by antibody and cells, there is still an 'intrinsic' protection of the surface membrane of the parasite at this stage.

The presence of host antigen determinants on the parasite surface may be the result of an active evasion mechanism by the schistosome, whereby it synthesizes molecules that mimic those of the host and serve as a disguise. Among the molecules recognized on parasites recovered from mice are the products of the major histocompatibility complex. Simpson (NIH, Bethesda) reported that monoclonal reagents indicate that an intact H-2 antigen is present on the schistosome surface. Although analysis of the parasite genome demonstrated some DNA sequence homology between S. mansoni and its hosts, genes coding for either human or mouse histocompatibility antigens were not present, so these molecules must be acquired from the host.

In vitro experiments (Rumjanek, NIMR, London) demonstrated that lipid composition of schistosomula was profundly affected by the presence of serum which is able to stimulate intense

exchange of lipids between the organism and the medium. Fetal calf serum produces a net depletion of lipids, whereas human serum produces a substantial uptake of cholesterol and triglycerides. This seruminduced lipid modulation correlates with the degree of protection observed in in vitro cytotoxicity assays and furthermore, no protection is afforded by incubation of schistosomula in delipidated serum. This strongly implicates lipids as being responsible for the acquisition of resistance by the parasite to the immune effector mechanisms. The protective effect of serum may be associated (Torpier) with low-density lipoproteins, which bind to the parasite surface, and this may be the basis of the lipid changes observed by Rumianek.

It is clear that our knowledge of the physiological functions of the schistosome DOM is still very limited, but in view of the importance that understanding of the membrane may have for the development of immuno-prophylactic and chemotherapeutic agents, it was felt that increased research efforts should be made to define the molecular structure of specific membrane proteins. The use of monoclonal antibodies and recombinant DNA technology to clone and sequence the parasite genes coding for these components is most likely to provide the way forward. The expression in microorganisms of genes coding for surface antigens may be an important step towards the production of significant amounts of parasite antigens for vaccination.

100 years ago

No group of animals is more characteristic of the peculiar fauna of Australia than the great family of Honey-Eaters (Meliphagidae), of which upwards of sixty species, belonging to many different genera, are distributed throughout the length and breadth of that Continent. But although the Honey-eaters are so common in Australia, and there is an extensive importation of living birds from Sydney and other Australian ports every year into this country, very few of the Meliphagidae have yet reached Europe alive. The fact is, that the organisation of the Honey-eaters, being adapted for an active and wandering life, in perpetual search of the nectar of the flowering-trees which their pencilled tongue so admirably fits them to collect, does not render them very suitable subjects



for captivity, and it is only recently that means have been found to preserve these birds alive and in good health in cages. It has thus happened that almost the only one of the vast tribe of Australian Honey-eaters that has been exhibited in the Zoological Society's aviaries is the present species, which we now figure (Fig. 18) from four examples lately received from New South Wales.

In his great work on the "Birds of Australia", Mr. Gould tells us that the Warty-faced Honey-eater is not only one of the handsomest of its tribe, but also one of the most beautiful birds inhabiting Australia, the strongly contrasted tints of its black and yellow plumage rendering it a most conspicouous and pleasing object, particularly during flight.

Although very generally distributed, its presence appears to be dependent upon the state of the Eucalypti, upon the blossoms of which it mainly depends for subsistence; it is consequently only to be found in any particular locality during the season that those trees are in blossom. It generally resorts to the loftiest and most fully-flowered tree, where it frequently reigns supreme, buffeting and driving every other bird away from its immediate neighbourhood.

The Lobed Muskduck (Biziura lobata). — Waterfowl have always formed a favourite portion of the Zoological Society's living collection, and it is with great satisfaction that every new addition to the already long list of "acclimatisable" Anatidae is announced in their journals. The species which is now portrayed (Fig. 19) is certainly one of the most remarkable that they have yet procured, and although perhaps not likely to be "acclimatised" at present, is well worth examination as being remarkable even amongst Australian animals, for several very abnormal features in its structure.

The Musk-duck has a lengthened, stiff, and leather-like appendage hanging from the under surface of the bill, and is the only member of the



family which possesses this singular structure. its lengthened tail, composed of twenty-four narrowed and stiffened feathers, is, no doubt, most serviceable to it in swimming and diving. The female does not carry the chin-lobe, and is very much smaller than the male bird.

male bird.

The Musk-duck is widely distributed on the Australian Continent, and also inhabits Tasmania. It frequents the bays and inlets of the sea, the upper parts of rivers, lakes, and secluded pools. "More than a pair are rarely seen at one time; often a solitary individual takes up its abode in some favourite pool, where it lives a life of complete seclusion, depending for its food and for its preservation from danger upon its powers of diving rather than upon those of flying. It is very difficult to shoot, as it dives instantly a gun is fired, so that the shot has hardly time to reach it.

From Nature 25, 608, 27 April, 1882.

REVIEW ARTICLE

Actin-binding proteins—regulators of cell architecture and motility

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Numerous actin-binding proteins from a variety of cell types have been described. Here I attempt to correlate the properties and functions of some of these. Three major classes have been identified: (1) cross-linking proteins which form filament bundles or isotropic gels; (2) proteins which cap filament ends and nucleate the polymerization of G-actin (many of these also sever actin filaments); (3) proteins which bind to G-actin and stabilize the monomer pool. Some of the proteins described here combine the properties of more than one class and the activities of many of them are regulated by changes in Ca^{2+} ion concentration.

ACTIN is a major constituent of many types of eukaryotic cells and is involved in a wide variety of motile processes including cell locomotion, cytoplasmic streaming and transport, secretion, phagocytosis and cytokinesis. 'Stress fibres', consisting of bundles of microfilaments1 are clearly seen in spreading or moving cells and these contain myosin, filamin, tropomyosin and α -actinin in addition to actin²⁻⁵. Actin is also concentrated as a dense filament meshwork in the cortical cytoplasm⁶, finger-like projections (filopodia) and veil-like sheets of membrane (lamellipodia or ruffles) display the dynamic activity of the cell surface during movement. This cortical cytoplasm undergoes dramatic changes in viscosity and stiffness which reflect the formation of actin filaments and their assembly into more highly organized meshwork structures. An understanding of cell motility therefore requires detailed knowledge of (1) the structural components involved with actin in the formation and rearrangement of this cortical network; (2) its biochemical composition; and (3) the mechanisms by which the interaction of these individual proteins are regulated.

Over the past five years much effort has been directed towards these goals⁸⁻¹¹. Many new actin-binding proteins have been identified, initially in crude cell-free extracts, but many of these have now been purified and partially characterized. Particular progress has been made with cytoplasmic gels isolated from macrophages and amoeboid cells and with the more highly organized structure of the intestinal microvillus.

Certain unifying principles are now emerging which may ultimately allow assignment of many of these apparently different proteins to a small number of functional groups. Just as the properties of myosins have been refined by natural selection to suit the particular requirements of a given muscle, so too proteins that form gel networks of actin or regulate the dissolution of these gels may have evolved specialized properties in their individual situations yet fall within a similar functional classification. The common theme is the role of actin filaments in maintaining cell architecture and generating movement. Actin-binding proteins provide the necessary variations for the part actin has to play in such widely differing processes as egg fertilization, blood clotting, intestinal absorption and tumour invasion.

Before reviewing these actin-binding proteins, it is important to note that while the roles of actin, myosin and associated proteins in striated muscle contraction may serve as models for certain types of cytoplasmic movement, there are basic differences between these two systems which demand distinctive properties for proteins common to both. The organization of filaments in vertebrate striated muscle is highly uniform and rigidly maintained to carry out its contractile functions, but in

non-muscle systems, filamentous structures are more dynamic, responding both temporally and spatially to the cell requirements. Ca^{2+} ions serve not only to promote acto-myosin interaction, but dissolve gels and in many cases sever actin filaments. For example, recent studies have shown that α -actinin from non-muscle cells cross-links actin filaments only in the absence of Ca^{2+} ions, in contrast to α -actinin in striated and smooth muscles, which shows no comparable Ca^{2+} -sensitivity. (Indeed if it were Ca^{2+} -sensitive, disintegration of the Z-disk structure might occur during muscle activation!)

General classification of actin-binding proteins

Actin is evolutionarily highly conserved in amino acid sequence¹² which probably reflects the strong selective pressure to preserve its three-dimensional structure and thus maintain essential interaction sites for many associated proteins. Central to the role of actin is its self-association from a monomeric form (G-actin, molecular weight (M_r) 42,000) to give polymers several micrometres in length, containing about a thousand monomers. This polymerization process has been studied in detail, particularly by Oosawa and Asakura¹³ and by Wegner¹⁴ and others but is still not fully understood. The rate of filament assembly is controlled by the formation of nuclei and their production is favoured in the presence of Mg²⁺ ions. Thus, at physiological KCl concentration and with MgCl₂ in excess of 1 mM, actin exists predominantly in the polymerized form, in equilibrium with a very small (<1 µM) concentration of free monomer (the so-called critical concentration). Monomers can add to both ends of growing filaments: Pollard and Mooseker¹⁵ estimated the rates of assembly and disassembly at each end and have shown that there is preferred assembly at the 'barbed end' while preferential disassembly occurs at the 'pointed end'. (Based on the structure of actin decorated with myosin subfragment-1, these ends have been termed 'barbed' and 'pointed' by analogy with the flights on an arrow.) A consequence of any difference in assembly rates at the two ends is that actin monomers can cycle through the filaments from barbed to pointed end; this has been termed 'treadmilling'16. ATP hydrolysis is required to maintain this steady-state process. Although treadmilling of tubulin in microtubules has been clearly shown ', actin monomer exchange in vitro appears to be limited in both extent and duration at actin concentrations >10 µM¹⁷ and steady-state treadmilling has not been demonstrated to date. One consequence of a difference in assembly rates at the two ends is that filament stability is altered if one end is blocked; for example, capping the preferred assembly

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end will cause monomer dissociation from the other end to establish a new monomer-polymer equilibrium¹⁸. I shall demonstrate below how capping proteins may affect both filament stability and polarity of assembly.

In many cell extracts, the concentration of monomeric actin is much higher than that expected on the basis of the critical concentration, due to the presence of profilins ¹⁹⁻²¹, proteins which bind specifically to G-actin and prevent polymerization. Profilins are representative of a class of proteins that stabilizes the actin monomer pool, although actin depolymerizing factors and even proteins such as DNase I may perform similar functions. (G-actin forms a 1:1 complex with DNase I and inhibits its enzymatic activity²².)

Once filaments have been formed, other proteins are needed to stabilize them in three-dimensional gels or oriented bundles. Several classes of cross-linking proteins have been described. Some of these 'gelation factors' provide cross-links insensitive to changes in Ca²⁺ ion concentration, while others operate only at Ca²⁺ concentrations $<10^{-7}$ M and the links are broken at $>10^{-6}$ M. Proteins that form actin bundles may also exist in either calcium-sensitive or calcium-insensitive forms.

Thus, these proteins act to form three-dimensional isotropic networks or tight bundles of actin filaments which themselves may further associate to produce more organized structures or to interact with membranes or cell organelles. As actin filaments are often found closely apposed to membranes, it is reasonable to suggest that specific linker proteins exist either as integral membrane components or to attach microfilaments to transmembrane elements. These have proved very difficult to identify.

Actin-containing gels can undergo contraction in conjunction with cytoplasmic myosins and suitable regulatory proteins (kinases and calmodulin). Another effect observed in the presence of Ca²⁺ ions is that of 'solation', where the gels are dispersed. Gel-sol transformations occur in some cases by severing of the actin filaments, and involve a new class of

actin-binding proteins which I term 'gelsolins'. In many cases, the severing protein remains bound to one end of the actin filament, preventing re-annealing, hence these proteins behave additionally as 'capping factors'. In this respect they mimic the effects of cytochalasin drugs, which inhibit elongation of actin filaments by binding to their barbed ends²³⁻²⁶. One possible outcome of such capping is an increased monomer concentration as a result of G-actin dissociation from the free pointed ends. In the presence of profilins, complete filament depolymerization may occur. These proteins have a second and rather different property. When added to G-actin in assembly conditions, they promote the formation of actin nuclei by binding to more than one monomer and thereby facilitate polymerization. Indeed both accelerated nuclei formation and decreased rates of elongation occur simultaneously with macrophage gelsolin in vitro (see below). Thus, depending on the conditions, these proteins may be involved in generating new filaments or in severing and disassembling pre-existing ones.

In the following sections I describe some of the proteins belonging to each of these classes, with particular emphasis on mammalian cells.

Actin cross-linking proteins—gelation and bundling

As already stated, there are several proteins which cross-link actin filaments to form either bundles or isotropic gels. In some cases, gel formation is favoured by a low molar ratio of a particular cross-linking protein to actin, while, with increasing amounts of the same cross-linker, parallel alignment of filaments occurs to form bundles. It is not possible, therefore, to designate every cross-linking protein within either the gelation or bundling subclass, but the large flexible proteins like filamin occur predominantly in gels, whereas much smaller proteins like fascin or fimbrin exist in organized actin bundles and are probably not sufficiently flexible to accommodate isotropic gel formation.

	Table 1 Clas	sification of actin-bindin	g proteins		
	Source	$M_r \times 10^{-3}$	No. of subunits	Ca ²⁺ - sensitivity	Ref.
Gelation proteins				· ·	
Filamin	Muscle	250	2	***	27
Filamin	Macrophage	270	2		31
Spectrin	Red blood cell	240 (α)]			31
		$220 (\beta)$	4	and the same	37
200	Sea urchin egg	220 (5)			40
********	Dictyostelium	120	***		60
Calatian and how the	,	120	***	name.	39
Gelation and bundling proteins α-Actinin					
α-Actinin α-Actinin	Muscle	105	2	****	43
	HeLa cells	100	2	+	49
Actinogelin	Ehrlich ascites	115	PR-	+	51
*****	Dictyostelium	95	ethan.	+	52, 53
9 81 3-	Acanthamoeba	85	_	+	54
Vinculin	Muscle	130	1	- vetera-	45
Vinculin	HeLa cells	130	1	+	50
Bundling proteins				•	30
Fascin	Sea urchin egg	£0			
Fimbrin	Microvillus	58 68		Insur	60
	Wicrovillus	08	1		62
Severing and capping proteins					
β-Actinin	Muscle	34 + 37	1 each		64
Gelsolin	Macrophage	90	1	+	66
Gelsolin	Platelets	90	1	+	69
Gelsolin	Plasma	90	î	+	73, 75
Villin*	Microvillus	95	î	+	73, 73 89, 91
Fragmin	Physarum	42	î	+	78, 79
	Dictyostelium	40	î		
Capping protein	Acanthamoeba	28 + 31	l each	+	81
Depolymerizing protein	Brain	19	i cacii		82
G-actin stabilizing proteins		**	****	stress	83
Profilin	I something of the				
Profilin	Lymphocytes	16	1	Ahmi	19
4 1 (1)(1)(1)	Acanthamoeba	14	1	Aller	21

This table includes the main proteins described in the text and omits a large number of well known actin-binding proteins found in muscle and elsewhere. The molecular weights are from polyacrylamide gel electrophoresis and as buffer conditions vary between different laboratories, the values cannot be strictly compared.

* Also be should be sho

* Also a bundling protein.

Filamins. A high molecular weight actin-binding protein, purified from smooth muscle, has been termed filamin²⁷. A structurally similar protein was identified in lung macrophages²⁸ and subsequently in neutrophils, platelets and other cell types. The general morphology of macrophage actin-binding protein is indistinguishable from filamin²⁹, both appearing as two flexible strands joined at one end, but they differ in their potencies for cross-linking actin³⁰. The overall length of the dimer of $M_r \sim 500,000$ is ~ 160 nm. Here I will use the term 'filamin' for this general class of large flexible actin cross-linking proteins.

Hydrodynamic measurements indicated a native M_r of 540,000 and macrophage filamin bound to actin filaments at 40 nm intervals, giving a stoichiometry of 1 filamin per 15 actin monomers at high molar ratios³¹. Bundles of actin filaments were seen in these conditions in vitro, but at lower ratios of filamin to actin, isotropic gels were produced. The concentrations of myosin required to produce contraction of actin gels were lower in the presence of macrophage filamin than in its absence³². When actin was polymerized in the presence of macrophage filamin, branched filaments were produced having an average length less than that seen in the absence of filamin³ Filamin also accelerates the onset of polymerization and produces a perpendicular branching of actin filaments, thereby promoting the formation of an isotropic gel. It is located in the pseudopods of polymorphonuclear leukocytes during locomotion and phagocytosis³⁴, in stress fibres and membrane ruffles of dividing chick embryo cells during interphase, and appears to be concentrated in the cleavage furrow during cytokinesis³⁵.

The morphological equivalent of filamin in the red blood cell is spectrin. Although spectrin appears as a loose, flexible and elongated dimeric molecule, in contrast to filamin, spectrin dimers are joined at both ends and self-associate end-to-end to form tetramers in 0.1 M KCl. Spectrin tetramers of ~200 nm in length cross-link small protofilaments of actin (10–15 subunits long on average) to form the scaffold of proteins that maintains the shape of the erythrocyte. These complexes of spectrin and actin are stabilized by a globular protein, band 4.1 in the Steck classification³⁶. This cytoskeletal framework is attached to the membrane via ankyrin, which links the spectrin tetramers to the main transmembrane protein, band 3 (see refs 37, 38 for reviews). Spectrin-like proteins may not be restricted to red blood cells, but may serve similar functions elsewhere.

Recently, a smaller protein that gels F-actin has been isolated from *Dictyostelium discoideum*^{39,40}. Like filamin, it promotes side-to-side and end-to-side interactions between actin filaments and increases the initial rate of actin polymerization. It also seems to be localized in the cell cortex and surface ruffles. Its native molecular weight and actin-binding stoichiometry have not been characterized.

α-Actinins. While filamin and spectrin are representatives of a class of large flexible actin cross-linking proteins, a distinct class of rod-like actin-binding proteins has been identified in muscle by Ebashi and co-workers⁴¹. The archetype, α-actinin, is located in the Z-disks of muscle and seems to be involved in cross-linking actin filaments⁴². The native protein is a dimer $(2 \times M_r 105,000)$ and the molecule is highly asymmetric, with a length of 400 Å (ref. 43). α-Actinin forms gels with F-actin in vitro, although its binding to actin is inhibited by tropomyosin⁴⁴.

In non-muscle cells, antibodies to muscle α -actinin stain stress fibres and regions close to the membrane ^{4,45}. These observations suggested that α -actinin is involved in binding microfilaments to membrane surfaces. However, although immunocytochemistry shows that α -actinin is concentrated close to focal adhesion plaques in cultured cells⁴⁵, it does not form a direct link with membranes⁴⁶. Furthermore, α -actinin can be selectively dissociated from plasma membranes with little parallel release of actin⁴⁷. α -Actinin therefore seems to act as a cross-linker and spacer between actin filaments⁴⁸.

An important recent discovery is the Ca^{2+} -sensitivity of α -actinin function. Both striated and smooth muscle α -actinins cross-link actin independently of Ca^{2+} concentration, but the

 α -actinin of HeLa cells forms actin gels only at Ca²⁺ ion concentrations $<10^{-7}$ M (refs 49, 50). This calcium-sensitive cross-linking is similar to that of actinogelin from Ehrlich ascites cells⁵¹. Rod-shaped, Ca²⁺-sensitive actin cross-linking proteins have also been identified in *Dictyostelium*^{52,53}, *Acanthamoeba*⁵⁴ and blood platelets. These proteins have not been completely characterized and although they may not be related by amino acid sequence or immunological criteria, their general morphology and actin cross-linking properties suggest that they may be classified within the same group. A number of smaller gelation factors have been described in amoeboid cells such as *Dictyostelium* and *Acanthamoeba*, based on viscometric or turbidimetric measurements, but these have yet to be purified.

One further interesting property of α -actinin is that it appears to dissociate the profilin-actin complex in vitro and promote polymerization to give cross-linked filaments⁵⁵. As 1 molecule of α -actinin per 200 profilactin molecules is sufficient to cause considerable polymerization, molecules such as α -actinin may serve to mobilize actin from non-filamentous pools in cells.

Vinculin. Another protein that cross-links actin filaments is isolated originally from smooth vinculin. muscle⁴⁵ Immunofluorescence showed that it is concentrated at the termini of stress fibres near the leading edges of cells and in the region of the adhesion plaques of cultured fibroblasts, but not in surface ruffles 45,56. Because detergents are not required to solubilize vinculin, it does not seem to be an integral membrane protein. However, as it is located where microfilaments terminate close to membranes⁵⁷, it may participate in anchoring microfilaments together at specific membrane sites. In contrast to α -actinin, vinculin reduces the low-shear viscosity of F-actin solutions ^{48,50}, apparently by forming bundles of tightly packed actin filaments in a paracrystalline manner ^{48,58}. This 'bundling' role of vinculin is not universally accepted 50. Furthermore. vinculin decreases the rate of polymerization of G-actin, which again contrasts with the behaviour of α -actinins.

One feature which vinculin shares with α -actinin is the Ca²⁺-sensitivity of its interaction with actin. Vinculin isolated from muscle shows no sensitivity to Ca²⁺ ion concentration, while the protein purified from HeLa cells (in two separable forms) causes a decrease in viscosity only at Ca²⁺ concentration >1 μ M (ref. 50). More information is needed to establish the function of this protein.

Fascin and fimbrin. In 1975, Kane⁵⁹ demonstrated that isotonic extracts of sea urchin eggs formed gels at low Ca^{2+} concentrations. One protein responsible is fascin $(M_r, 58,000)$, which binds to actin to form needle-like structures composed of parallel arrays of filaments with cross-bridges at 11 nm intervals⁶⁰. This interaction is not sensitive to Ca^{2+} ions. Addition of another component of M_r 220,000 from the original gel causes aggregation of the needles into a three-dimensional network. Immunofluorescence studies show that fascin is localized in the microvillar cores which form after fertilization of the sea urchin eggs⁶¹. Thus it may be involved directly in microvillus organization and morphogenesis.

Intestinal brush border microvilli also contain proteins which stabilize the core microfilament bundles. Antibodies to one of these proteins, fimbrin, also stain membrane ruffles, microspikes and microvilli in several types of cultured cells⁶². Fimbrin binds F-actin at a saturation level of 1 fimbrin per 2-3 actin monomers, to form tightly packed bundles⁶³. In native microvilli there is a second actin cross-linking protein, villin, which will be considered in detail later. These two proteins act in a concerted manner to form filament bundles when mixed together with actin at molar ratios approximating those in isolated core bundles, that is, villin/fimbrin/actin monomers = 1:1:10.

Actin severing, capping and depolymerizing proteins

 β -Actinin and gelsolins. The earliest evidence for a protein that reduced the viscosity of actin filament solutions came from the work of Maruyama. He subsequently purified this protein,

which he called β -actinin, from a muscle extract. Gel filtration suggested a native M_r of $\sim 70,000$ and two bands of M_r 34,000 and 37,000 were obtained on SDS gels. β -actinin was shown to inhibit actin filament growth and re-annealing but to facilitate polymerization of G-actin⁶⁴. These two apparently contradictory properties will become a recurring theme (see below). Subsequently, several proteins that disrupt actin gels have been found in both mammalian and amoeboid systems.

Yin and Stossel⁶⁵ identified gelsolin, a calcium-dependent regulatory protein that solated actin-containing gel extracts from macrophage cytoplasm. Gelsolin is a globular monomeric protein of M_r 91,000, with two calcium-specific binding sites $(K_d = 9.2 \times 10^{-7} \text{ M})^{66}$. Evidence obtained from viscometry, flow birefringence and electron microscopy indicates that at low molar ratios to actin, gelsolin rapidly cleaves the actin filaments into smaller pieces⁶⁷. It was proposed on the basis of classical polymer theory⁶⁸ that this fragmentation would profoundly affect the network properties of actin. Wang and Bryan⁶⁹ described a platelet protein of M_r 90,000 which increased the rate of nuclei formation from G-actin and blocked monomer addition to the barbed ends of subfragment-1 (S-1)-decorated filaments.

Taken together, these observations suggest the existence of a severing protein which caps filament ends to prevent reannealing, but is also capable of complexing two or more actin monomers in polymerizing conditions to nucleate assembly. Recent studies with macrophage gelsolin have demonstrated very clearly this dual function ⁷⁰. Elongation of pre-formed actin nuclei in the presence of G-actin was slowed down, whereas polymerization was facilitated in the absence of such nuclei. Electron microscopy showed preferred growth from the pointed ends when gelsolin was added to S-1-decorated actin filament fragments. Thus gelsolin binds to both G- and F-actin and in both cases an increased number of shorter filaments is produced.

Several workers have reported the presence of an actin-depolymerizing factor in blood plasma⁷¹⁻⁷³. The purified protein has a molecular weight of 90,000 (refs 74, 75). Recent experiments suggest that this protein is very similar if not identical to gelsolin, in respect of its immunological cross-reactivity⁷ and its fragmentation patterns on gel electrophoresis (B. Pope, unpublished results). Furthermore, the protein behaves like gelsolin in accelerating actin polymerization in the presence of Ca2+ ions (ref. 75 and H. E. Harris and A.W., unpublished results). Increasing the concentration of plasma gelsolin (within a molar ratio to actin monomers of 1:200 to 1:50) produced filaments of decreasing length and gel electrophoresis showed most of the gelsolin sedimenting with the F-actin⁷⁵. We have shown that this protein releases depolymerized actin from filaments very rapidly and in a calcium-independent manner 77 Furthermore, a complex of the plasma protein with one or two actin monomers has been identified (H. E. Harris, unpublished results). These results cannot be reconciled within a model in which the sole action of gelsolin on actin filaments is severing, followed by capping of the barbed ends. The paradox may be reconciled partly by differences in the experimental conditions and by the fact that gelsolin binds to both G- and F-actin, but it is premature to speculate a detailed model for the function of this protein.

Fragmin and related proteins. Proteins that sever actin filaments have been isolated from several amoeboid cells. Fragmin is a protein which severs actin filaments and nucleates polymerization of monomers in a calcium-sensitive manner^{78,79}. It also forms a stable dimer with G-actin, but this has no severing activity. Tropomyosin or heavy meromyosin binding protect against fragmentation of filaments⁸⁰. A protein having similar properties has been purified from *Dictyostelium*⁸¹ and *Acanthamoeba* contains a capping protein that prevents gel formation by binding to the barbed ends of filaments, although this does not seem to have any severing activity⁸². A much smaller depolymerizing protein has been purified from brain, but its kinetics of actin disassembly are rather different from that of gelsolin⁸³.

Villin. Villin deserves special attention because it has both actin-severing and bundling activities, combining the properties of gelsolin and α -actinin. Villin is the major actin-associated protein in the core bundles of microfilaments from microvilli of intestinal epithelial cells. These microvilli are $\sim 1-2~\mu m$ long and each contains 20–30 actin filaments, held together as core bundles by villin and fimbrin. The bundles are attached to the inner membrane surface by cross-connections with a periodicity of 33 nm (see ref. 84 for review). Another protein $(M_r 110,000)^{8.5}$ forms the cross-connections to the membrane surface at 33-nm spacings, while the remaining protein component in demembranated cores is calmodulin $^{86.87}$.

Villin has two distinct properties in its interaction with F-actin. In the absence of Ca^{2+} ions ($<10^{-7}$ M), it cross-links actin filaments into bundles, but when the Ca2+ concentration is raised above 10^{-6} M, not only are the bundles dispersed, but the filaments are also fragmented. These dual properties of villin have been intensively studied ⁸⁷⁻⁹³. In addition, villin binds to actin-DNase I complexes in the presence of Ca2+ ions but is eluted from these complexes when the calcium is removed. This provides rapid purification of the protein⁸⁹. The paradox that villin does not bind to actin-DNase in the absence of calcium but bundles F-actin is not understood, but may reflect differences in its binding properties with monomeric and polymeric actin. Complexes of villin/actin/DNase (1:2:2) have been isolated by sucrose density gradient centrifugation⁸⁷. The presence of two actin monomers bound to villin suggests that it may nucleate actin filament assembly of G-actin: both villin and villin-actin complexes accelerate the rate of filament formation 90,91, the villin remaining attached to the filaments at their barbed ends90. These reactions occur only in the presence of Ca^{2+} ions (villin has one calcium-binding site, $K_d = 2.5 \times$ 10⁻⁶ M; ref 87). Addition of calcium chelators to villin-severed filaments causes re-annealing⁹², suggesting that villin capping activity is lost when calcium is removed.

Selective proteolysis by V-8 protease cleaves villin into a core fragment of M_r 90,000 and a headpiece of M_r 8,000⁹⁴. The core fragment behaves rather like gelsolin—it promotes assembly of G-actin and severs filaments, but no longer bundles F-actin 90,94. By contrast, the headpiece binds F-actin independently of Ca2+ concentration and competitively inhibits F-actin bundling by villin95. It is not clear whether bundling requires a dimer of villin, but this seems unlikely because villin does not self-associate in solution89. In addition, it is unknown how calcium ions disrupt bundling and instead cause severing and capping. If, as expected, both headpiece and core domains are involved in actin bundling, the interaction of headpiece must be modulated by Ca2+ ions. The severing mechanism is totally obscure; it could occur by direct intercalation into filaments or by weakened actin-actin interactions resulting from lateral attachment of villin to F-actin. The physiological function of the calcium-sensitive, actin-severing activity of villin is obscure, but may be related to the vesiculation of microvilli that occurs in nutritional stress and in other pathological conditions93, and a role for the large amount of calmodulin found in microvilli may be to prevent this vesiculation from occurring during normal uptake of Ca2+ ions.

The actin bundles in the microvilli extend into the terminal web, where they seem to be interconnected by fibrillar structures. In addition there are interconnections to a band of actin filaments encircling the cells close to the zonula adherens. Myosin and tropomyosin are concentrated near the rootlets of the microvillar bundles 96,97 , with tropomyosin and α -actinin being concentrated in the region of the zonula adherens 46 . The terminal web also contains a complex meshwork of 10-nm filaments which may serve to maintain rigidity of the microvilli and the adherence to adjacent epithelial cells.

Regulatory mechanisms

Table 1 summarizes information about the different classes of actin-binding proteins discussed here. It is clear that Ca²⁺ ions regulate the formation of actin bundles or gel networks in two

distinct ways. Cross-links formed by α -actinins are broken when the Ca2+ concentration rises above micromolar levels, but rapidly reform when calcium is removed. Actin gels are also disrupted in the presence of calcium by gelsolins, but in this case, removal of the calcium does not restore the gel network immediately as re-annealing of filaments occurs at a slower rate⁶⁷. In addition, Ca²⁺ ions independently regulate myosin filament formation and acto-myosin interaction via a calmodulin-dependent myosin light-chain kinase98

Calcium transients can therefore cause both contraction and solation. In some situations these phenomena may occur together, with localized disruption of gels and microfilaments sliding past one another as in muscle⁵². However, much greater contractile force may be produced if the gel network contracts without solation99. Information is now needed to establish the precise Ca2+ concentrations required for these different processes and the extent to which they are independent or synchronized.

Calcium ions also regulate the interaction of gelsolins and other capping proteins with both monomeric actin and with the ends of pre-existing filaments. The function of these capping activities in cells is unclear. Based on the behaviour of these proteins in vitro, I suggest two different properties. In conditions where steady-state treadmilling of actin may occur, capping the barbed ends of filaments would be expected to facilitate disassembly of monomers from their pointed ends 18. This could lead to complete depolymerization if the monomers were complexed with profilin or taken up by other actin filaments. Alternatively, capping proteins may function, together with free monomers, to promote actin filament formation with preferred polarity and thereby control the localization of microfilaments in cells. But how could this be achieved? One possibility is that capping factors may themselves bind to membrane proteins directly.

Actin filament growth in vivo may occur predominantly from the barbed ends¹⁰⁰. Assembly from stabilized monomer pools can be extremely rapid: the formation of the acrosomal process in Thyone sperm is the best example of this 101. How is this triggered? Current evidence suggests that a transient proton flux may initiate acrosome formation 102. The state of the nonpolymerized actin in Thyone sperm is as yet unclear, but it is likely that a profilin-like protein stabilizes the monomer pool as has been demonstrated elsewhere 19-21. In platelets it has been shown that activation by thrombin is correlated with actin filament formation¹⁰³ and this process can be inhibited by cytochalasin drugs, which also prevent the morphological changes that normally occur after platelet activation1

Our understanding of the organization and interactions of various actin-binding proteins with actin filaments in striated muscle, both during development and in active contraction, owes much to the highly organized arrangements of these proteins and their assemblies in the sarcomeres. Similar ultrastructural analysis is being carried out on the intestinal microvillus, which also is a stable and well-organized structure having relatively few components. In motile systems, however, structures like contractile rings or pseudopodia are probably too labile for X-ray analysis and may be too poorly organized for three-dimensional image reconstruction from electron micrographs. The mechanisms by which contractile elements are moved and relocated in cells, for example in cytokinesis or cytoplasmic streaming, remain a mystery: it seems probable that macromolecular structures are not moved intact, but require disassembly and reassembly. Specific organizing centres may exist, such as are known for microtubules.

Research over the past five years has identified various actinbinding proteins in a number of cell models. Here I have attempted to group these into a simple classification. Biochemical analysis is progressing rapidly, but it is important now to investigate the roles of these components in their cellular locations. One obvious approach involves microinjection 105, for example, using suitably labelled actin-binding proteins or antibodies to these components where they already exist in cells. In this way we may be able to characterize particular motile events in relation to specific proteins. If these motile events are triggered by transient fluxes of Ca2+ or other ions, then fluorescent or chromophoric reagents can be used to explore the localization and kinetics of these ion movements. In addition, the target proteins (for example, calmodulin in the case of calcium) must be identified and their subsequent activities established. One obvious regulatory mechanism involves protein phosphorylation, but direct binding by the target protein may also modulate the properties of actin-binding proteins. Knowledge of the molecular events and regulatory mechanisms involved in cell motility is a major scientific challenge and may prove of considerable medical importance.

I thank Professor J. A. Spudich and his colleagues at the Department of Structural Biology, Stanford University for much stimulating discussion during a sabbatical year. I also thank Professors K. Burridge, J. Condeelis, B. Jockusch, E. Korn, M. Mooseker, T. Pollard, T. Stossel, L. Tilney and K. Weber for providing preprints.

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UV radiation from the young Sun and oxygen and ozone levels in the prebiological palaeoatmosphere

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UV measurements of young T-Tauri stars, resembling the Sun at an age of a few million years, have recently been made with the International Ultraviolet Explorer. They indicate that young stars emit up to 10⁴ times more UV than the present Sun. The implications for the origin and evolution of O_2 and O_3 in the prebiological palaeoatmosphere are presented here. The results of photochemical calculations indicate that the O_2 surface mixing ratio was a factor 10^4 – 10^6 times greater than the standard value of 10⁻¹⁵. This new value reconciles the simultaneous existence of oxidized iron and reduced uranium.

A RELIABLE picture of the environment of the early Earth depends critically on the correct quantification of the amount of solar radiation impinging on the Earth. Standard stellar evolutionary models predict that 4,500 Myr ago, the Sun's luminosity was lower than today by $\sim 30\%$ (ref. 1). This has created the 'dim Sun paradox' because such dimming translates into a decrease of the Earth's effective temperature, T_* , of ~8%, sufficient to keep T_* below the freezing point of seawater for ~2,000 Myr (ref. 2). On the other hand, there is evidence of liquid water as early as 3,500 Myr ago², so a paradox arises. Explanations proposed to solve the problem include either increasing the greenhouse efficiency²⁻⁴ or varying the Earth's early albedo⁵. In either case, one makes inferences about the

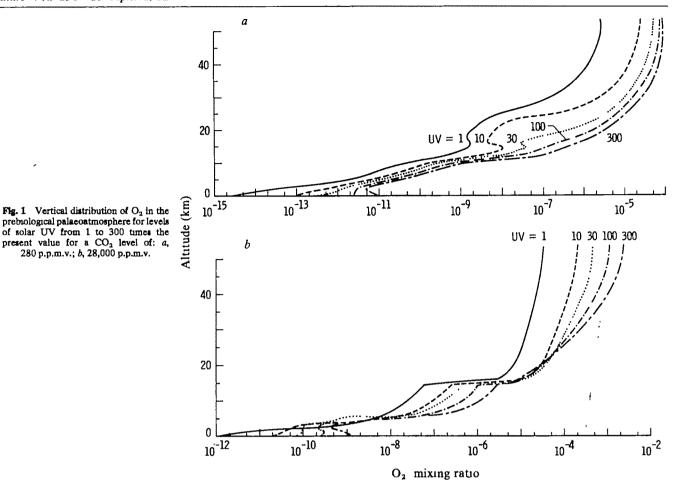
physical parameters characterizing the palaeoatmosphere using astronomical data.

We consider here another astronomical input, the UV radiation from the Sun which had a great impact on the early atmosphere of the Earth 6-12. In fact, UV radiation initiated the photochemical processes that led to the formation of oxygen (O₂) and ozone (O₃) in the prebiological palaeoatmosphere. By virtue of its strong absorption in the UV (200-300 nm), ozone protects life at the surface of the Earth from this lethal radiation. Hence, the evolution of ozone, and its variation over geological time had very important implications for the biological evolution of primitive organisms⁶⁻¹². An accurate quantification of the levels of oxygen and ozone in the prebiological

Table 1 Ratios of stellar to solar UV line fluxes intercepted at the distance of the Earth								
λ(A)	Identity	T Tau	DR Tau	RW Aur	GW Ori	CoD-35°10525	RU Lup	S CrA
1,240	Νv	2.5×10 ⁵		$<4.0\times10^{3}$	2.3×10 ⁵	6 1×10 ⁴	2.4×10^{3}	
1,304	OiSí	13×10 ⁵		9.4×10 ³	9.9×10 ⁴	3 0×10 ⁴	6.5×10^{3}	
1,335	Сп	7.8×10 ⁴		9.6×10^{2}	4.3×10 ⁴	17×104	1 9×10 ⁴	
1,400	Sirv	2.2×10 ⁵	5.5×10^4	1.2×10^4	2.3×10 ⁵	2.0×10^4	3 3×10 ⁴	2 4×10 ⁴
1,550	Crv	1.4×10^{5}	8 8×10 ⁴	3.2×10^{3}	1.5×10^{5}	2.9×10 ⁴	1.2×10 ⁴	8.1×10 ³
1,640	Не п	1.4×10^{5}	5.9×10 ⁴	$< 1.5 \times 10^3$	2.0×10 ⁵	2.6×10 ⁴	5 8×10 ³	≤7.7×10³
1,813	Si II, S i	2.9×10^4	1.9×10^4		1.1×10 ⁴	2.8×10³	5.1×10^{3}	56×10 ³

The radii of the stars have been taken as (in solar radii) 6 8, 4 7, 3.1, 8.4, 3.4, 2.6, and 3.2, respectively.

280 p.p.m.v.; b, 28,000 p.p.m.v.



palaeoatmosphere and the level of UV radiation reaching the surface of the Earth are therefore important when considering biological evolution on Earth.

The stellar evolutionary models cannot be used to predict the amount of UV generated by the young Sun and one must, therefore, rely on direct observational data. Because until recently such data were available only for the present Sun, estimates for the past UV radiation were based either on the assumption that the lower solar luminosity also implied a lower UV component or else it was assumed that the UV radiation was the same in the past as it is today⁶⁻¹².

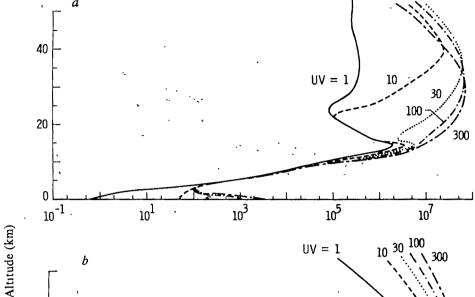
Recent observational data gathered through the International Ultraviolet Explorer (IUE) have, however, indicated that neither approach is correct. Young stars have been found to emit considerably more UV radiation than the present Sun.

The T-Tauri stars are very young stars with masses roughly equal to the Sun's. As such, they are considered to resemble the Sun at an age of a few million years. Several T-Tauri stars have recently been studied in the UV with IUE¹³⁻¹⁷. The primary result of these observations is that the stars emit strongly in the UV. Most noticeable are the chromospheric emission lines arising from the hot (10⁴-10⁵ K) atmospheres of the stars. The surface fluxes of the lines, (defined to be the flux emitted per unit stellar surface area), have been determined to be 100-5,000 times greater among the T-Tauri stars than in the Sun¹⁸. In addition, these protostars are still contracting and thus are larger than the Sun. Thus, the UV flux intercepted at the distance of the Earth is increased by an additional factor of $(R_{\star}/R_{\odot})^2$ due to the greater surface area of the protostar. We have computed the ratio of stellar-to-solar UV flux as would be measured at the Earth's distance for seven T-Tauri stars, given in Table 1. Radii for these stars were estimated from temperatures and luminosities of the stars largely taken from ref. 19. The values range from 3 to 8 solar radii and are given in Table 1. We find that the UV line flux from the T-Tauri stars is 10³-10⁴ times the present solar value.

The emission lines dominate the spectrum between 100 and 200 nm. At longer wavelengths the UV continuum of the T-Tauri stars dominates. The continuum fluxes are also enhanced by a few hundred times relative to the Sun. As we will discuss below, the wavelength intervals discussed here cover the maximum in the UV absorption cross-section of H₂O, CO₂, O_2 , and O_3 .

Young stars which are older than the T-Tauri stars continue to emit strongly in the UV, although not at the extreme levels of the T-Tauri stars. UV emission-line surface fluxes decrease with age approximately as $t^{-0.5}$ to $t^{-0.9}$ (ref. 20). The age dependent dence for the UV surface fluxes resembles that of Ca II emission, determined previously by ground-based near-UV observations²²⁻²³. We have computed the enhancement of the UV flux intercepted at the distance of the Earth as a function of age for a solar-type star, using the observational relationship reported in ref. 20 corrected for the changing radius of the contracting star. Radii at specific ages for solar mass stars were estimated from temperatures and luminosities in theoretical H-R diagrams¹⁹. The results are given in Table 2. We find that, even after the star has reached the main sequence $(5 \times 10^7 \text{ yr})$, begins

Table 2	Stellar UV flux as a function of age		
Age (yr)	UV enhancement		
10 ⁶	10 ⁴		
10 ⁷	500		
5×10^{7}	100		
10*	32		
5×10 ⁸	8		
10°	4		
5×10°	1		



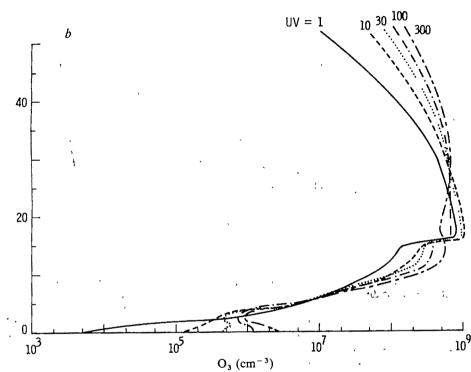


Fig. 2 Vertical distribution of O₃ in the prebiological palaeoatmosphere for levels of solar UV from 1 to 300 times the present value for a CO₂ level of: a, 280 p.p.m.v.; b, 28,000 p.p.m.v.

hydrogen burning and becomes stable, it emits about 100 times the UV radiation presently observed in the Sun.

There is no reason to believe that the youth of the Sun differed from that indicated by the T-Tauri and other young stars. We, therefore, conclude that the UV output of the young Sun was 10-1,000 times its present value. The high level of UV radiation thus indicated must have a profound effect on our understanding of the early atmosphere of the Earth.

Photochemistry of oxygen and ozone

Enhanced levels of UV radiation that may have been emitted from the Sun in its early stages may have had some very important implications for the origin and evolution of the early atmosphere, particularly for prebiological levels of oxygen (O_2) and ozone (O_3) . In the prebiological palaeoatmosphere the formation of O_2 was initiated by solar UV radiation, that is, the photolysis of water vapour (H_2O) , accompanied by the exospheric escape of atomic hydrogen (H), and the photolysis of carbon dioxide (CO_2) . The photochemical reactions governing these processes are:

$$H_2O + h\nu \rightarrow OH + H; \lambda \le 240 \text{ nm}$$
 (1)

and,

$$CO_2 + h\nu \rightarrow CO + O; \lambda \le 230 \text{ nm}$$
 (2)

Table 3 Photodissociation reactions and diurnally-averaged rates at 53.5 km for a latitude of 30° (equinoctial conditions) (for present atmosphere)

		Destruction
No.	Reaction	(cm ⁻³ s ⁻¹)
J1 '	$O_2 + h\nu \rightarrow O + O$	1.41×10 ⁶
J2	$O_1 + h\nu \rightarrow O + O_2$	4.40×10^{6}
J3	$O_1 + h\nu \rightarrow O(^1D) + O_2$	7.07×10^{7}
J4	$H_2O + h\nu \rightarrow OH + H$	5.32×10^{2}
J5	$N_2O + h\nu \rightarrow O(^1D) + N_2$	1.29×10^{2}
J6	$HNO_3 + h\nu \rightarrow OH + NO_3$	2 63×10°
37	$NO_7 + h\nu \rightarrow O + NO$	1.33×10 ⁴
18	$H_2O_2 + h\nu \rightarrow OH + OH$	2.52×10^{2}
19	$HNO_2 + h\nu \rightarrow OH + NO$	4 31×10 ¹
J10	$NO_3 + h\nu \rightarrow NO + O_2$	7.85×10^{-1}
J11	$NO_3 + h\nu \rightarrow NO_2 + O$	7.85×10^{0}
J12	$N_2O_5 + h\nu \rightarrow NO_2 + NO_3$	5.21×10 ⁻⁴
J13	HCl+hv.→H+Cl	$1.47 \times 10^{\circ}$
J14	$CIO_2 + h\nu \rightarrow CIO + O$	6.41×10 ⁻³
J15	$CIO + h\nu \rightarrow CI + O$	1.45×10^{2}
J16	$Cl_2 + h\nu \rightarrow Cl + Cl$	1.43×10 ⁶
J17	$CINO_3 + h\nu \rightarrow CIO + NO_2$	4.77×10 ⁻⁴
J18	$CCl_4 + h\nu \rightarrow 2Cl + products$	1.27×10^{-5}
J19	$CH_1CI + h\nu \rightarrow CH_1 + CI$	′ 1.09×10 ⁻² ·
J20	$CH_2O + h\nu \rightarrow H + HCO$	4.00×10^{1}
J21 '	$CH_2O + h\nu \rightarrow H_2 + CO$	4.15×10^{1}
J22	$CH_3CCl_1 + h\nu \rightarrow Cl + products$	1 20×10 ⁻⁵
J23	$CH_3OOH + h\nu \rightarrow CH_3O + OH$	1.82×10^{0}
J24	NO+hv→N+O	9.12×10^{1}
J25	$CO_2 + h\nu \rightarrow CO + O$	4.51×10^{-1}

Table 4 Chemical reactions

		Rate constant
No.	Reaction	$(cm^3 s^{-1} or cm^6 s^{-1})$
1	$O + O_2 + M \rightarrow O_3 + M$	$1.1 \times 10^{-34} \exp(510/T)$
2	$O + O_3 \rightarrow 2O_2$	$1.5 \times 10^{-11} \exp(-2.218/T)$
3	$O(^{1}D) + O_{2} \rightarrow O + O_{2}$	$3.2 \times 10^{-11} \exp(67/T)$
4 5	$O(^{1}D) + N_{2} \rightarrow O + N_{2}$ $N_{2}O + O(^{1}D) \rightarrow 2NO$	$1.8 \times 10^{-11} \exp(107/T) 6.6 \times 10^{-11}$
6	$N_2O + O(^1D) \rightarrow N_2 + O_2$	5.1×10^{-11}
7	$NO+O+M \rightarrow NO_2+M$	$1.6 \times 10^{-32} \exp(584/T)$
8	$NO + O_3 \rightarrow NO_2 + O_2$	$2.3 \times 10^{-12} \exp(-1.450/T)$
9 10	$NO_2 + O \rightarrow O_2 + NO$ $NO_2 + O_3 \rightarrow NO_3 + O_2$	$9.3 \times 10^{-12} 1.2 \times 10^{-13} \exp(-2,450/T)$
		•
11 12	$NO + HO_2 \rightarrow NO_2 + OH$ $NO_2 + OH + M \rightarrow HNO_3 + M$	$3.5 \times 10^{-12} \exp(250/T)$
13	$HNO_3 + OH \rightarrow NO_3 + H_2O$	$1.5 \times 10^{-14} \exp(650/T)$
14	$H_2O + O(^1D) \rightarrow 2OH$	2.2×10^{-10}
15 16	$ \begin{array}{c} H + O_2 + M \rightarrow HO_2 + M \\ H + O_3 \rightarrow OH + O_2 \end{array} $	$2.1 \times 10^{-32} \exp(290/T)$ $1.4 \times 10^{-10} \exp(-470/T)$
17	$OH + O \rightarrow H + O_2$	$2.3 \times 10^{-11} \exp(110/T)$
18	$OH + O_3 \rightarrow HO_2 + O_2$	$1.6 \times 10^{-12} \exp(-940/T)$
19 20	$OH + OH \rightarrow H_2O + O$ $HO_2 + O \rightarrow OH + O_3$	$4.5 \times 10^{-12} \exp(-275/T)^{\circ}$ 4.0×10^{-11}
21 22	$HO_2 + O_3 \rightarrow OH + 2O_2$ $HO_2 + OH \rightarrow H_2O + O_2$	$1.1 \times 10^{-14} \exp(-580/T) 4.0 \times 10^{-11}$
23	$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$	2.5×10^{-12}
24	$H_2O_2 + OH \rightarrow HO_2 + H_2O$	$2.7 \times 10^{-12} \exp(-145/T)$
25 26	$OH + NO + M \rightarrow HNO_2 + M$ $NO + NO_3 \rightarrow 2NO_2$	2.0×10 ⁻¹¹
27	$O(^{1}D) + N_{2} + M \rightarrow N_{2}O + M$	3.5×10^{-37}
28	$O(^{1}D) + H_{2} \rightarrow OH + H$	9.9×10^{-11}
29 30	$O(^{1}D) + CH_{4} \rightarrow OH + CH_{3}$ $NO_{2} + O + M \rightarrow NO_{3} + M$	$1.4 \times 10^{-10} \\ 1.0 \times 10^{-31}$
31 32	$NO_2 + NO_3 \rightarrow N_2O_5$ $N_2O_5 \rightarrow NO_2 + NO_3$	$1.5 \times 10^{-13} \exp(861/T) 1.2 \times 10^{14} \exp(-10,319/T)$
33	$N_2O_5 + H_2O \rightarrow 2HNO_3$	1.0×10^{-20}
34	$N_2O_5 + O \rightarrow 2NO_2 + O_2$	3.0×10^{-16}
35 36	$N+NO_2 \rightarrow N_2O+O$ $N+O_2 \rightarrow NO+O$	$2.1 \times 10^{-11} \exp(-800/T)$ $4.4 \times 10^{-12} \exp(-3,220/T)$
37	$N+NO \rightarrow N_2+O$	3.4×10^{-11}
38	$N+O_3 \rightarrow NO+O_2$	1.0×10^{-15}
39 40	$CI + O_3 \rightarrow CIO + O_2$ $CIO + O \rightarrow CI + O_2$	$2.8 \times 10^{-11} \exp(-257/T) 7.7 \times 10^{-11} \exp(-130/T)$
	•	
41 42	$ClO + NO \rightarrow Cl + NO_2$ $Cl + CH_4 \rightarrow HCl + CH_3$	$6.5 \times 10^{-12} \exp(280/T) 9.6 \times 10^{-12} \exp(-1,350/T)$
43	Cl+H ₂ →HCl+H	$3.5 \times 10^{-11} \exp(-2.290/T)$
44	$Cl + HO_2 \rightarrow HCl + O_2$	4.8×10 ⁻¹¹
45 46	$Cl+H_2O_2 \rightarrow HCl+HO_2$ $Cl+HNO_3 \rightarrow HCl+NO_3$	$1.1 \times 10^{-12} \exp(-980/T) 1.0 \times 10^{-11} \exp(-2,170/T)$
47	Cl+CH ₂ O→HCl+HCO	$9.2 \times 10^{-11} \exp(-68/T)$
48	HCl+OH→Cl+H ₂ O	$2.8 \times 10^{-12} \exp(-425/T)$
49 50	$HCl+O \rightarrow Cl+OH$ $Cl+O_2+M \rightarrow ClO_2+M$	$1.1 \times 10^{-11} \exp(-3.370/T)$
51		$2.7 \times 10^{-9} \exp(-2,650/T)$
-52	$ClO_2 + M \rightarrow Cl + O_2 + M$ $Cl + ClO_2 \rightarrow 2ClO$	8.0×10^{-12}
53	$Cl + ClO_2 \rightarrow Cl_2 + O_2$	1.4×10^{-10}
54 55	$OH + OH + M \rightarrow H_2O_2 + M$ $H_2O_2 + O \rightarrow OH + HO_2$	* $2.8 \times 10^{-12} \exp(-2.125/T)$
56	$OH + CH_4 \rightarrow CH_3 + H_2O$	$2.3 \times 10^{-12} \exp(-2.123/T)$ $2.4 \times 10^{-12} \exp(-1.710/T)$
57	$CIO + NO_2 + M \rightarrow CINO_3 + M$	*
58 59	$O + CINO_3 \rightarrow CIO + NO_3$ $O(^1D) + HC1 \rightarrow OH + CI$	$3.0 \times 10^{-12} \exp(808/T)$ 1.4×10^{-10}
60	H ₂ +OH→H ₂ O+H	$1.2 \times 10^{-11} \exp(-2,200/T)$
61		*
62	$CH_3 + O_2 + M \rightarrow CH_3O_2 + M$ $CH_3O_2 + HO_2 \rightarrow CH_3OOH + O_2$	$7.7 \times 10^{-14} \exp(1,300/T)$
63	$CH_3O_2 + NO \rightarrow CH_3O + NO_3$	7.4×10^{-12}
64 65	$CH_3O + O_2 \rightarrow CH_2O + HO_2$ $CH_2O + OH \rightarrow HCO + H_2O$	$9.2 \times 10^{-13} \exp(-2,200/T)$ 1.0×10^{-11}
66	CH ₂ O→OH+HCO	$3.0 \times 10^{-11} \exp(-1.550/T)$
67	$HCO + O_2 \rightarrow CO + HO_2$	5.0×10^{-12}
68 69	$CO + OH \rightarrow H + CO_2$ $CH_3Cl + OH \rightarrow Cl + products$	* 1.8 x 10 ⁻¹² exp(-1.112/ <i>T</i>)
70	$CH_3CI+OH \rightarrow CI+products$ $CH_3OOH+OH \rightarrow CH_3O_2+H_2O$	$1.8 \times 10^{-12} \exp(-1,112/T)$ $2.1 \times 10^{-12} \exp(-145/T)$
71	$OH + CH_3CCl_3 \rightarrow Cl + products$	$5.4 \times 10^{-12} \exp(-1.820/T)$
72	$O+O+M\rightarrow O_2+M$	$2.8 \times 10^{-34} \exp(710/T)$
73 74	$H+H+M \rightarrow H_2+M$	8.3×10 ⁻³³
75	$H_2+O \rightarrow OH+H$ $H+HO_2 \rightarrow O_2+H_2$	$3.0 \times 10^{-14} \exp(-4,480/T)$ $4.7 \times 10^{-11} (\times 0.29)$
76	$H + HO_2 \rightarrow H_2O + O$	$4.7 \times 10^{-11} (\times 0.02)$
77	$H + HO_2 \rightarrow OH + OH$	$4.7 \times 10^{-11} (\times 0.69)$

^{*} See ref. 34.

Reaction (1) leads to the production of oxygen atoms (O) through:

$$OH + OH \rightarrow O + H_2O \tag{3}$$

The oxygen atoms formed in reactions (2) and (3) form molecular oxygen (O₂) through the following reactions

$$O + O + M \rightarrow O_2 + M \tag{4}$$

$$O + OH \rightarrow O_2 + H \tag{5}$$

The destruction of O_2 is controlled by its photolysis and reaction with molecular hydrogen (H_2) resulting from volcanic missions:

$$O_2 + h\nu \rightarrow O + O; \lambda \le 242 \text{ nm}$$
 (6)

and

$$2H_2 + O_2 \rightarrow 2H_2O \tag{7}$$

(See reactions (19) and (74) in Table 4.)

Closely coupled to the origin and evolution of O_3 is the origin and evolution of O_3 . O_3 is photochemically produced through the reaction:

$$O + O_2 + M \rightarrow O_3 + M \tag{8}$$

Table 5 Column density of O₃ in the prebiological alaeoatmosphere for various combinations of atmospheric CO₂ and solar UV flux

Solar UV flux	CO ₂	O ₃ column (cm ⁻²)
1*	1†	1.72×10^{12}
	10	2.49×10^{14}
	100	1.38×10^{15}
10	1	3.94×10^{13}
	10	5.36×10^{14}
	100	1.95×10^{15}
30	1	1.50×10^{14}
	10	7.81×10^{14}
	100	2.06×10^{15}
100	1	1.76×1014
	10	8.15×10^{14}
	100	2.04×10 15
300	1	1.52×1012
	10	9.41×10 ¹⁴
	100	2.19×10 ¹⁵

^{*} Present value of solar UV flux²⁷. Other values epresent multiples of present value.

 \mathbf{O}_3 is photochemically destroyed through photolysis and reaction with $\mathbf{O}:$

$$O_3 + h\nu \to O_2 + O; \ \lambda \le 1,100 \text{ nm}$$
 (9)

and

$$O_3 + O \rightarrow O_2 \tag{10}$$

In addition, O_3 is photochemicaly destroyed by a series of catalytic cycles involving the oxiles of nitrogen $(NO + NO_2)$, hydrogen $(OH + HO_2)$, and chlome (Cl + ClO).

To study the effects of enhaned solar UV radiation on the levels of O_2 and O_3 in the prebidogical palaeoatmosphere, the photochemical model of the plaeoatmosphere of Levine et al.²⁴ was modified. This model which was originally developed to study the effect of anthropgenic perturbations on future

 $[\]dagger$ Pre-industrial level of CO $_2$ (p.p.m.v.). Other values represent multiples of this value.

levels of O₃, has been used to study the origin and evolution of O₃ for specified levels of palaeoatmospheric O₂ ranging from 10⁻⁴ of the present atmospheric level (PAL) to the present atmospheric level (1 PAL)²⁴. The model was modified such that O₂, CO₂, and H₂ which were previously specified as input parameers are now considered as chemically-active species, whose vertical profiles are calculated using coupled species continuity-flux equations, which contain both chemical production and loss terms and vertical eddy transport²⁵. The inclusion of O₂, CO₂, and H₂ as chemically-active species makes it a total of 31 species whose vertical profiles are solved simultaneously by coupled species continuity-flux equations. The other calculated speces are: O₃, O, N₂O, N, NO, NO₂, NO₃, N₂O₅, HNO₂, HNO₃, H₂O, H, OH, HO₂, H₂O₂, CH₄, CO, CH₃OOH, CH₂O, CH₃, HCC, CH₃O₂, CH₃O, Cl, ClO, HCl, CH₃Cl, and ClNO₃. Very short lived species assumed to be in instantaneous photochemical equilibrium include: O(1D), ClO2, and Cl2. The model now include 25 photochemical processes listed in Table 3, and 77 chemica reactions listed in Table 4. The US Standard Atmospheric Mid-Latitude Spring/Autumn temperature and H₂O profiles are specified in the troposphere. A primordial temperature profile that decreases linearly from the tropopause to the mesoshere is used. This profile is based on coupled photochemica-radiative equilibrium temperature calculations in the O₃-deficent palaeoatmosphere²⁶. While H₂O is specified in the tropospiere, it is calculated through the continuity-flux equation for F₂O in the stratosphere. Water soluble species, for example, INO3, H2O2, CH3OOH, HCl, ClO, and ClNO3 are lost throughout rainout, with a rainout coefficient of 1.0× 10⁻⁶ s⁻¹. The model extends from the surface up to 53.5 km with the tropopase at a height of 14.5 km. Photodissociation rates are diurnaly-averaged for a latitude of 30° and solar declination of 0°. The model includes the solar spectrum from 110 to 735 nm (ef. 27). Further details about the model are given in refs 24, 25.

For the present alculations, we have incorporated the boundary conditions for O2 and H2 given in ref. 28. O2 is considered as a chemically-active species with a zero flux lower boundary condition and H₂ hs a prescribed surface mixing ratio of 17 p.p.m.v. (ref. 28). We have studied the sensitivity of prebiological O₂ and O₃ to the level of CO₂ by performing calculations for the pre-industria CO₂ value (280 p.p.m.v.), and for 10-100 times this value. Voltile outgassing scenarios suggest that the early atmosphere may have contained as much as three orders of magnitude more CO₂ than found in the present atmosphere ^{29,30}. According to these scenarios, the bulk of the early CO2 went into the ocean and eventually formed sedimentary carbonates. The outgassed CO2 on both Mars and Venus had a very different fate-remaining in the atmospherepresumably due to the lack of liquid water and life on these planets. For our calcultions we have used five values for the flux of solar UV: the pesent value²⁷ and multiples of 10, 30, 100, and 300 times the resent value.

Received 28 December 1981, acceptd 23 February 1982

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Oxygen and ozone in the prebiological palaeoatmosphere

O₂ profiles for the pre-industrial level of CO₂ (280 p.p.m.v.) and 100 times this value are given in Fig. 1. For each CO2 level calculations have been performed for five values of solar UV radiation ranging from 1 to 300 times the present value. The calculated O₂ profile for contemporary values of CO₂ and UV exhibits a very strong altitude dependence, with a minimum O_2 mixing ratio of $<10^{-14}$ at the surface and a maximum O_2 mixing ratio of about 2×10^{-6} above 40 km, in excellent agreement with earlier calculations²⁸. For a CO₂ value of 280 p.p.m.v. (Fig. 1), we found that increasing the UV flux from the present value to 300 times the present value resulted in increasing the surface mixing ratio of O_2 from $<10^{-14}$ to $\sim10^{-11}$. The O_2 maximum between 40 and 50 km increased from a mixing ratio of $\sim 2 \times 10^{-6}$ to 8×10^{-5} as the solar UV flux was increased by a factor of 300. For a CO₂ value of 100 times the pre-industrial value (Fig. 1b), increasing the UV flux by a factor of 300 increased the surface mixing ratio of O₂ from 10⁻¹² to 10⁻⁹. and increased the O_2 maximum at ~ 50 km from $\sim 3 \times 10^{-5}$ to $\sim 2 \times 10^{-3}$. The corresponding O₃ calculations are shown in Fig. 2a (for $CO_2 = 280$ p.p.m.) and Fig. 2b (for $CO_2 = 100$ times greater). For a CO₂ value of 280 p.p.m.v. (Fig. 2a), increasing the UV flux by a factor of 300 increased the surface concentration of O_3 from 1 to $\sim 3 \times 10^3$ cm⁻³ and increased both the height of the O_3 maximum from 14.5 to ~ 30 km and the peak concentration from 1×10^6 to $\sim 5 \times 10^7$ cm⁻³. For the enhanced value of CO₂ (Fig. 2b), increasing the UV flux by a factor of 300 increased the surface concentration of O_3 from 5×10^3 to 2×10^6 cm⁻³. For this level of CO₂ and for all values of enhanced UV radiation the height of O₃ maximum was found to be between 15 and 20 km with a concentration that varied between 5 and $10 \times 10^8 \,\mathrm{cm}^{-3}$. The total O₃ column (molecules cm⁻²) above the surface of the Earth for the O₃ profiles shown in Fig. 2 and for a CO₂ value of 10 times the pre-industrial value is summarized in Table 5.

Conclusion

Our calculation indicates that the surface mixing ratio of O₂ increased by a factor of 104-106 times the standard value of 10⁻¹⁵ (depending on the assumed value of CO₂) for enhanced level of UV radiation associated with the early Sun. Corresponding enhancement of O₃ level was also found.

These increased levels of O2 and O3 may have had important implications for early geology and on the origin and evolution of life. While the details of such implications are beyond the scope of this article, note that the higher value of O_2 found here falls within the lower (10^{-13}) and upper (10^{-3}) O_2 values arrived at to explain the simultaneous existence of oxidized iron and reduced uranium on the early Earth³¹⁻³³

V.M.C. and J.S.L. thank Dr K. M. Towe for his helpful suggestions. C.L.I. thanks Dr M. S. Giampapa for assistance.

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Chemical Kinetic and Photochemical Data for Use in Stratospheric Modelling JPL Publ 81-3 (Jet Propulsion Laboratory, Pasadena, 1981)

Sr and Nd isotope geochemistry of oceanic basalts and mantle evolution

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Sr and Nd isotope ratios are reported for 17 mid-ocean ridge basalts and for 11 oceanic islands and island groups. Data from the Azores, Samoa and the Society Islands diverge significantly from the mantle array. These results are not explained by binary mixing of depleted and undepleted mantle reservoirs or by variable magmatic depletion of a planetary reservoir, but support mantle evolution models involving re-injection of crust material into the mantle.

ISOTOPIC studies of volcanic rocks have greatly enhanced knowledge of the chemistry and evolution of the Earth's mantle. Because of the lesser possibility of crustal contamination, much effort has focused on oceanic volcanics. In general, Sr and Nd isotope ratios in such rocks have proved to be well correlated. The establishment of this linear correlation, or 'mantle array', has been the basis of quantitative models for the present-day chemical structure and evolution of the mantle¹⁻⁴, and for estimates of the planetary ⁸⁷Sr/⁸⁶Sr and Rb/Sr ratios. New data reported here reveal that basalts from some islands have Sr and Nd isotope ratios which deviate significantly from the mantle array. These data thus raise doubts about the validity of some of these models and the estimated Rb/Sr ratio of the Earth. On the other hand, the new data generally support mantle evolution models involving significant transfer of crustal material into the mantle.

Samples from the East Pacific Rise, Mid-Atlantic Ridge, Kerguelen, Samoan Islands, Society Islands, Hawaiian Islands, Easter Island, Sala y Gomez, Guadalupe, Tristan de Cunha, Gough, St Helena and the Azores (Fig. 1) were analysed for isotopic composition of Sr and Nd at the Department of Terrestrial Magnetism, Washington; US Geological Survey, Denver; and Max-Planck-Institut (MPI) für Chemie, Mainz. Conventional mass spectrometric and chemical techniques were used except for some strontium analyses performed at MPI, which were analysed using a double collector system installed in a Finnigan-MAT 261 mass spectrometer. Replicate analysis of samples and standards confirmed that there was no systematic difference between values obtained using single collector and those obtained using double collector. The double collector mode does, however, produce better run statistics in a shorter analysis time. Some samples used in this study have previously been analysed for 87Sr/86Sr but have been reanalysed because of the higher analytical precision presently obtainable.

Most of the basalts analysed in this study are young—less than 1 Myr old—and hence the measured ratio is the initial ratio. The exceptions are basalts from Kerguelen and the Societies. No age corrections have been applied to these data as exact ages are not known in all cases. The Kerguelen samples range in age up to 27 Myr (ref. 5). In these cases, the measured ⁸⁷Sr/⁸⁶Sr ratios exceed the initial ratios by small, but significant, amounts (up to 0.00010). This difference does not affect the interpretation of the data. In the case of the Society Islands samples, which range in age up to 4 Myr (ref. 6), age corrections are not significant (equal to or less than analytical error).

Results are reported in Table 1 and Fig. 2. We first discuss the data in the context of individual localities before considering their more general implications.

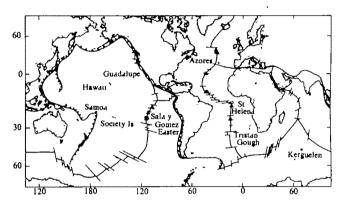


Fig. 1 Plate tectonic map of the world showing locations of dredged basalts

(▲) and islands included in this study

Kerguelen

This island was considered possibly to be a continental fragment but its oceanic nature is now firmly established^{5,7,8}. Dosso et al.^{7,8} have found that basaltic rocks from Kerguelen have ¹⁴³Nd/¹⁴⁴Nd ratios, implying source histories ranging from slight light-rare-earth depletion to significant light-rare-earth enrichment (slightly positive to significant negative ε_{Nd}). Samples analysed in this study come principally from Foch Island and Corbet Peninsular. Seven of these fall within the range of values reported by Dosso et al., but three, all from Foch Island, have significantly lower ⁸⁷Sr/⁸⁶Sr and higher ¹⁴³Nd/¹⁴⁴Nd ratios than those reported by Dosso. Kerguelen thus encompasses an enormous range of Sr and Nd isotope ratios and includes basalts with Nd isotope ratios both significantly higher and lower than bulk earth. This implies notable mantle heterogeneity on the scale of tens of kilometres. That Foch Island basalt KG5-3 has Sr and Nd isotope ratios similar to basalts from the remainder of Kerguelen but radically different from the flows immediately overlying and underlying it⁵ is further evidence of small-scale heterogeneity.

Hawaii

Sr and Nd isotopic data reported here fall entirely within the range of values reported by O'Nions et al.⁹. Our data, and those of O'Nions et al. do not reveal any systematic differences in isotopic composition between tholeiitic and alkalic lavas or systematic inter- or intra-island variations, that is while ⁸⁷Sr/⁸⁶Sr and ¹⁴³Nd/¹⁴⁴Nd ratios are quite variable in Hawaii, these variations appear to be largely random. This may, however, be due to the limited amount of data. Two apparent exceptions are (1) basalts from Molakai have notably higher ⁸⁷Sr/⁸⁶Sr than other islands and (2) the Honolulu series volcanics of Oahu have less radiogenic Sr than the Waianae series

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Table 1 All data reported relative to values of 87Sr/86Sr = 0.70800* for the E&A standard and 143Nd/144Nd = 0.51265* for the BCR-1 standard

Table 1	All data reported relat	tive to values of 87	$Sr/^{86}Sr = 0.70800*$	for the E&A standard and $^{143}Nd/^{144}Nd = 0$).51265* for the BCR-1	standard
Mid-Atlantic Ridge		⁸⁷ Sr/ ⁸⁶ Sr	¹⁴³ Nd/ ¹⁴⁴ Nd	Samoan Islands	⁸⁷ Sr/ ⁸⁶ Sr	¹⁴³ Nd/ ¹⁴⁴ Nd
HU-197	45°39' N 27°52' W	0.70288 ± 6	0.513109 ± 35	Manu'a Islands		
CH43 106-16 39,13	45°11' N 27°54' W	0.70338 ± 5	0.513101 ± 20	\$17	0.70469 ± 3	0.512785 ± 8
CH43 106-17 39	45°11′ N 27°54′ W	0.70307 ± 4	0.513049 ± 30	S221a	$\int 0.70444 \pm 3$	0.512837 ± 7
CH43 106-18 39,13	45°11′ N 27°54′ W	0.70337 ± 4	0.513086 ± 20		0.70441 ± 3	0.512831 ± 15
	(leached)	0.70319 ± 5		Tutuila	`	
CH43 104-7	45°11′ N 27°54′ W	0.70326 ± 4	0.513054 ± 40	TUT-9 20	0.70500 ± 2	0.512805 ± 18
CH43 104-59 39	45°11′ N 27°54′ W	0.70314 ± 5	0.513096 ± 35	TUT-6D ²⁰ (unleached)	0.70518 ± 3	0.515060.51
HU-56-2 CH13 PD-11 13	45°13′ W 28°00′ W 50°44′ N 29°52′ W	0.70308±4	0.513087 ± 20	(leached) TL-13 ²⁰	0.70517 ± 2	0.512860 ± 21
A150 RD-20 39,13	30°01′ N 39°04′ W	0.70264 ± 6 0.70263 ± 6	$\begin{array}{c} 0.513216 \pm 25 \\ 0.513235 \pm 20 \end{array}$	ST-26 ²⁰	0.70505 ± 1 0.70475 ± 3	0.512782 ± 21 0.512816 ± 18
AD2-1 40,41	20°40′ S 13°16′ W	0.70205 ± 0 0.70285 ± 4	0.513253 ± 20 0.513157 ± 19	Upolu	0.1041515	0.312610±16
AD3-3 40,41	5°47′ S 11°25′ W	0.70235 ± 2	0.513291 ± 11	UF-1 19	$\int 0.70490 \pm 3$	0.512857 ± 21
AD5-5 40,41	9°39' N 40°27' W	0.70265 ± 4	0.513141 ± 12		0.70493 ± 3	0.512871 ± 17
AD5-11 40,41	9°39' N 40°27' W	0.70284 ± 3	0.513163 ± 11	UF-2 19	0.70500 ± 4	0.512935 ± 9
				UP-1 19 (unleached)	$\int 0.70583 \pm 1$	0.512699 ± 13
East Pacific Rise					0.70584 ± 1	0.512705 ± 21
Amph D-4 15	18°52′ S 113°19′ W	0.70257 ± 2	0.513107 ± 10	US-1 19 (leached)	0.70582 ± 2	0.512702 ± 8
PD1-1 41,42 PD3A 41,42	7°47′ S 108°10′ W	0.70251 ± 5	0.513211 ± 17		0.70583 ± 3 $\int 0.70562 \pm 2$	0.512746 ± 14 0.512678 ± 17
PD4G 41,42	12°52′ S 110°57′ W 18°25′ S 113°20′ W	0.70252 ± 2 0.70253 ± 2	0.513202 ± 20 0.513163 ± 11	UM-2 19	0.70562 ± 2	0.512678 ± 17 0.512669 ± 27
1.040	10 23 3 113 20 44	0.70233 1 2	0.515105 ± 11	UPO-2 20 (unleached)	0.70486 ± 2	0.512784 ± 14
Hawaiian Islands				(leached)	0.70483 ± 2	0.512821 ± 15
Loihi Seamount				UPO-9 20	0.70547 ± 2	0.512750 ± 8
Dredge 2		0.70350 ± 2	0.512979 ± 14	UPO-F-9-1 20 (unleached)	0.70494 ± 2	0.512905 ± 20
Hawaii				(leached) UPO-F-9-8 ²⁰	0.70491 ± 2	0.512922 ± 20
BHVO-1 Kila	auea 1919	0.70346 ± 2	0.512948 ± 13	JKU-1 ²⁰	0.70540 ± 1 0.70552 ± 1	0.512796 ± 16 0.512615 ± 24
7777 44 II 77. I		0.70345 ± 5	0.512990 ± 24		0.70565 ± 1	0.512615 ± 24 0.512655 ± 10
KH-11 11 Kol	hala	0.70354 ± 2	0.512979 ± 32	JKU-21 ²⁰	0.70559 ± 1	0.512633 ± 10 0.512642 ± 15
MG-1a 11 Kol	hala	0.70357 ± 2 0.70361 ± 2	0.512988 ± 32 0.512973 ± 30	Savai'i		
	hala	0.70358 ± 2	0.512973 ± 13 0.512973 ± 13	VA-1 19 (unleached)	$\int 0.70635 \pm 2$	0.512775 ± 18
Molakai					0.70636 ± 2	0.512763 ± 17
GA 572 43		0.70382 ± 4	0.512944 ± 10	(leached)	0.70635 ± 2	0.512761 ± 8
WMOL-1 22		$0.70411 \pm 2\dagger$	0.512943 ± 18	VM-2 19	0.70651 ± 2	0.512734 ± 16
Oahu (Waianae Ser	ries)	0.000000 . 41	0.544044	VP-1 19	$\begin{cases} 0.70601 \pm 3 \\ 0.70602 \pm 2 \end{cases}$	0.512689 ± 12 0.512694 ± 16
5X-667-3 ²² 5X-197-2 ²²		$0.70378 \pm 2 \pm 0.70380 \pm 2$	0.513036 ± 24 0.512982 ± 10	- 10	0.70575 ± 2	0.512054 ± 16 0.512750 ± 14
GA553 43		0.70380 ± 2 0.70381 ± 3	0.512982 ± 10 0.512935 ± 11	VP-2 ¹⁹ (unleached)	0.70576 ± 2	0.512777 ± 18
GA557 43		0.70361 ± 6	0.512986 ± 9	(leached)	0.70577 ± 2	0.512757 ± 22
Kauai				SAV-1 ²⁰	0.70573 ± 3	0.512729 ± 20
GA565 43		0.70371 ± 4	0.512973 ± 9	Society Islands		
GA649 43		0.70359 ± 4	0.513019 ± 28	Tahiti 74-416 ¹⁸	0.70434 ± 2	0.512850 ± 8
ID-872-2 ²²		$0.70369 \pm 3\dagger$	0.513026 ± 34	74-422 ¹⁸ (unleached)	0.70434 ± 2 0.70419 ± 2	0.512830 ± 8 0.512931 ± 20
Nihoa 8G141-2 ²²		0.70364 ± 3†	0.513068 ± 20	(leached)	0.70418 ± 2	0.512918 ± 18
La Perouse Pinnacle	es	0.70304±31	0.515008 ± 20	Hauhine		
LPP-W-30 ²²		0.70371 ± 4	0.513014 ± 9	73-95 18 (unleached)	0.70567 ± 2	0.512704 ± 14
				(leached)	0.70563 ± 1	0.512674 ± 8
Kerguelen				Bora Bora 73-332 ¹⁸	0.70495 ± 1	0.512829 ± 17
Foch Island				Maupiti	0.70493 x 1	U.312029 # 17
KG2-1 5		0.70388 ± 5	0.512878 ± 30	73-204 18	0.70530 ± 1	0.512706 ± 10
KG4-1 ⁵		0.70407 ± 6	0.512870 ± 30	Tahaa		
KG5-3 ⁵		$\begin{cases} 0.70532 \pm 2 \\ 0.70530 \pm 2 \end{cases}$	0.512631 ± 24 0.512641 ± 12	73-185 18 (unleached)	0.70691 ± 2	0.512587 ± 26
KG6-2 5		0.70398 ± 4	0.512041 ± 12 0.512907 ± 25	(leached)	0.70694 ± 2	0.512571 ± 16
Rest of Island		***************************************	***************************************	73-186 ¹⁸ (unleached)	0.70504 ± 2	0.512804 ± 36
KG8-4 (unleach		0.70531 ± 4	0.512645 ± 40	(leached) Gough Island	0.70505 ± 2	$0-512803 \pm 20$
(leached	i)	0.70534 ± 6	0.510/#1 .50	G-8 15	0.70532 ± 6	0.512574 ± 20
KG11-1 5		0.70526 ± 6	0.512671 ± 30 0.512631 ± 35	Tristan da Cuhna		
KG14-3 ⁵ 175		0.70532 ± 5 0.70538 ± 4	$0.512631 \pm 35 \\ 0.512685 \pm 20$	Tr-1 15,45 (NMNH 110014)	0.70505 ± 2	0.512534 ± 10
1380		0.70531 ± 6	0.512657 ± 25	Tr-4 15,45 (NMNH 110017)	0.70509 ± 2	0.512526 ± 15
1381		0.70521 ± 6	0.512671 ± 15	Tr-6 15,45 (NMNH 110019) St Helena	0.70501 ± 2	0.512567 ± 10
				2928 ^{15,46}	0.70287 ± 3	0.512883 ± 18
Easter Island				2876 ⁴⁶ (NMNH 99659)	0.70287 ± 3 0.70287 ± 2	0.512863 ± 18 0.512853 ± 29
17732 44 (Poike)		0.70326 ± 5	0.512992 ± 30	2893 46 (NMNH 99661)	0.70292 ± 2	0.512843 ± 32
EC-307 44 (Poike		0.70319 ± 6	0.513062 ± 25	2926 46 (NMNH 99653)	0.70285 ± 2	0.512871 ± 32
EH-39 ⁴⁴ (Poike) EH-20 ⁴⁴ (Rano I		0.70328 ± 4 0.70321 ± 5	0.513056 ± 25 0.513004 ± 30	2882 ⁴⁶ (NMNH 99658)	0.70292 ± 1	0.512857 ± 12
KK72-43-7 44		0.70321 ± 3 0.70324 ± 4	0.513004 ± 30 0.513012 ± 35	102 (NMNH 109984)	0.70296 ± 2	0.512842 ± 14
EC-385 44 (Terev	aka)	0.70324 ± 5	0.513012 ± 30 0.513008 ± 30	Azores Sao Miguel		
, , , , , , , , , , , , , , , , , , , ,	•			SM-2A 16	0.70337 ± 6	0.512926 ± 30
Sala y Gomez				SM-12 16	0.70514 ± 1	0.512720 ± 30 0.512707 ± 11
Y73-4-30-20 44		0.70327 ± 5	0.512953 ± 20	SM-6 ¹⁶	$\int 0.70430 \pm 2$	0.512812 ± 10
					0.70429 ± 2	0.512810 ± 18
Guadalupe		0.70224	0.510040 : 00	SM-28 16	0.70525 ± 6	0.512732 ± 28
GU77 15		0.70326 ± 4	0.512942 ± 25	Faial F-2 16	0.70386 ± 2	0.512871 ± 24
				F-14 ¹⁶	0.70386 ± 2 0.70394 ± 6	0.512871 ± 24 0.512870 ± 28
				F-21 16	0.70394 ± 6 0.70384 ± 6	0.512970 ± 25 0.512943 ± 25
				F-29 16	0.70392 ± 2	0.512877 ± 18
				F-33 ¹⁶	0.70393 ± 6	0.512843 ± 24

^{*} Measured values at the Max-Planck-Institut. These values were 0.70792 and 0.51271, and 0.70808 and 0.51273 at the Department of Terrestrial Magnetism, Washington and the US Geological Survey, Denver respectively. Error quoted are 2 s.e.m. based on in-run statistics. This statistic probably slightly overestimates reproducibility, which is better evaluated from the replicate analyses. Superscripts refer to other published information of the sample.

† Sr data from ref. 22.

of that island. These distinctions do not apply to Nd. On Hawaii, the individual volcanoes are not isotopically distinct, although the Kohala lavas are notably isotopically uniform, in agreement with Feigenson *et al.*¹⁰. The easternmost and youngest volcano of the chain, Loihi, has Sr and Nd isotope ratios typical of the chain in general.

East Pacific Rise and Islands

The East Pacific Rise, Easter Island, Sala y Gomez and Guadalupe all have similar isotopic compositions which fall within the range of values from the Galapagos¹¹. The Poike and Ranu Kau basalts from Easter Island exhibit variations only slightly greater than analytical error, while EC-385 of the Terevaka volcanics has a significantly lower ⁸⁷Sr/⁸⁶Sr ratio. Previously reported ⁸⁷Sr/⁸⁶Sr ratios from Easter (summarized in ref. 12) show much more dispersion, perhaps due to the relatively large analytical error of the earlier data. Samples from the East Pacific Rise have isotopic compositions similar to other mid-ocean ridge basalts (MORB).

Mid-Atlantic Ridge and South Atlantic Islands

Except for those samples from the vicinity of 45°N, the Mid-Atlantic Ridge samples have typical MORB compositions. The 45°N area was known to be anomalous from previous stdies¹ The relatively high 87Sr/86Sr and lower 143Nd/144Nd ratios of the 45°N samples are thus not surprising. Note that the ⁸⁷Sr/⁸⁶Sr ratios of CH43 106-18 were lower after HCl leaching, indicative of seawater contamination. This may also account for the high 87Sr/86Sr ratio of CH43 106-16. Three new analyses from Tristan da Cunha fall within the range of values previously reported^{9,14}, and although there are still relatively few data from this island, it appears that the volcanics are isotopically homogeneous. Gough Island appears to be isotopically similar to but distinct from Tristan. Both Tristan and Gough have ¹⁴³Nd/¹⁴⁴Nd ratios lower than chondritic, implying timeintegrated light-rare-earth enrichment of their sources. The six St Helena samples analysed in this study suggest this island also has an isotopically uniform source but one which is quite distinct from Tristan and Gough (A. Zindler, personal communication, however, reports samples with distinctly higher ⁸⁷Sr/⁸⁶Sr). The St Helena data fall off the mantle array and are unique in that they plot to the left of it. St Helena is also unique in having extremely high ²⁰⁶Pb/²⁰⁴Pb ratios¹⁵.

Azores

Most Azores islands basalts have ⁸⁷Sr/⁸⁶Sr ratios between 0.70332 and 0.70354 but basalts from Sao Miguel can have ratios up to 0.70525 (ref. 16). Hawkesworth et al. ¹⁷ found that ⁸⁷Sr/⁸⁶Sr and ¹⁴³Nd/¹⁴⁴Nd ratios from Sao Miguel define a trend which is distinct from the mantle array. New data reported here confirm the results of Hawkesworth et al. Faial, which after Sao Miguel has the highest ⁸⁷Sr/⁸⁶Sr in the Azores, has isotopic compositions which plot at the intersection of the Sao Miguel trend and the mantle array.

Society Islands

Duncan et al.^{6,18} found that these islands become progressively older to the west, ranging in age from 1.0 to 4.3 Myr and that their Sr is highly radiogenic with ⁸⁷Sr/⁸⁶Sr ratios of 0.7036–0.7067. ⁸⁷Sr/⁸⁶Sr and ¹⁴³Nd/¹⁴⁴Nd ratios fall on the trend of the Sao Miguel data but extend it to much higher ⁸⁷Sr/⁸⁶Sr and lower ¹⁴³Nd/¹⁴⁴Nd ratios. Sample 73–185 from Tahaa, at one extreme of this trend, deviates markedly from the mantle array. There is no apparent systematic variation either in time or in space, the island of Tahaa exhibiting more variation than the other islands combined.

Samoa

Like basalts from Sao Miguel and the Society Islands, many Samoan basalts have ⁸⁷Sr/⁸⁶Sr and ¹⁴³Nd/¹⁴⁴Nd ratios which

plot off the mantle array. However, unlike Society and Sao Miguel basalts, Samoan basalts do not form a linear trend but scatter about the Sao Miguel-Society trend. Hedge et al. ¹⁹ found that ⁸⁷Sr/⁸⁶Sr ratios increased westwards along the island chain. This is borne out by the new data, which include many of the samples analysed by Hedge et al. The geographical variation is not strong, however, as, for example, Tutuila basalts plot entirely within the Upolu field.

Samoan volcanism has occurred in two phases^{20,21}: the earlier, or pre-erosional volcanism is normal island-chain type, that is becoming progressively younger to the east, but a younger episode of post-erosional volcanism has occurred along a fissure connecting the three westernmost and oldest islands. Both pre-and post-erosional volcanics have Sr and Nd isotope ratios which plot off the mantle array, and there is no apparent difference in the extent to which the two types deviate from the mantle array, although the post-erosional lavas generally have the highest ⁸⁷Sr/⁸⁶Sr and lowest ¹⁴³Nd/¹⁴⁴Nd ratios. Basalts from the Manua islands, at the eastern end of the chain, plot within the mantle array.

Contamination during or after magma ascent

Possible causes for the divergence of isotope ratios of some of the islands from the mantle array include post-eruptional weathering effects and contamination by altered basalt or sediment of the oceanic crust during magma ascent. Acid-leaching, which is useful in identifying samples affected by post-eruptional alteration (see, for example, ref. 8), reveals no difference between leached and unleached fractions (Table 1). This argues persuasively against the former possibility. Arguments against the latter have been given by Duncan and Compston¹⁸ and by Hawkesworth *et al.*¹⁷. To these may be added the following observation. The Samoan basalts tend to be primitive in nature (that is, high Mg and Ni)19, which suggests they did not spend any considerable time in a crustal magma chamber and hence are particularly unlikely to have suffered crustal contamination. Although oceanic crustal contamination during magma ascent cannot be ruled out entirely, it is unlikely to be the cause of the observed isotopic variations, which are considered to reflect chemical and isotopic heterogeneity in the mantle.

Intra-island and intra-archipelago variations

Figure 2 illustrates the variability of isotopic heterogeneity of oceanic islands. Single isolated islands such as St Helena, Tristan da Cuhna, Ascension and Bouvet (Table 1 and ref. 9) tend to

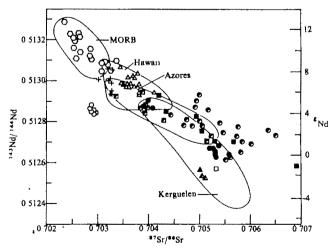


Fig. 2 Results obtained in this study plotted on a ⁸⁷Sr/⁸⁶Sr versus ¹⁴³Nd/¹⁴⁴Nd variation diagram. Fields for mid-ocean-ridge basalts (MORB), Azores, Hawaii and Kerguelen are drawn on the basis of these and previously published data ○, MORB; +, Easter Island and Sala y Gomez; *, Guadalupe; △, Hawaii, ○, St Helena; El, Azores, ●, Samoa, Societies, □, Gough, △, Tristan da Cunha, and ♠, Kerguelen Much of the data from Samoa fall outside the boundary of the conventional mantle array and data from Sao Miguel (Azores) and the Societies define a trend at low angle to the mantle array.

be isotopically homogeneous while islands or island groups associated with large oceanic plateaus, such as Iceland, Kerguelen, Hawaii, the Galapagos and the Azores (Table 1 and refs 8, 9, 11, 17, 22, 23), exhibit more variation, as do the Pacific island chains, such as Hawaii, Samoa and the Societies.

However, the apparent homogeneity of some islands may be related to sampling problems, and the question of local isotopic variations on oceanic islands requires a much more systematic approach to sampling than has previously been undertaken.

These intra-island and intra-archipelago variations may result partially or largely from mixing of an enriched source (or a melt derived from such a source) with depleted mantle. The apparent homogeneity of some islands indicates that such mixing is not ubiquitous. Furthermore, in the case of Samoa, Sr-Nd and particularly Sr-Pb relationships²⁴ suggest heterogeneity of the source is due at least in part to factors other than mixing with depleted mantle.

Mantle evolution

Explanations for the Nd-Sr relationship which have been suggested include mixing between a primitive or planetary reservoir having $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ of $\sim\!0.705$ and $^{143}\mathrm{Nd}/^{144}\mathrm{Nd}$ of $\sim\!0.51265$ and a depleted reservoir with the isotopic characteristics of MORB (that is $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}=0.7027$ and $^{143}\mathrm{Nd}/^{144}\mathrm{Nd}=0.51315)$ and variable depletion of an initially homogeneous planetary reservoir, the most severely depleted portion represented by the MORB reservoir $^{2-4}$. A basic assumption of these models is that the relationship between Sr and Nd isotopic ratios in oceanic basalts is monotonic. That the $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ ratio of the bulk earth may be found by taking the intersection of the 'mantle array' with the chondritic $^{143}\mathrm{Nd}/^{144}\mathrm{Nd}$ is implicit.

The new data presented here and those of Hawkesworth et al. 17 clearly violate the assumption of a single linear relationship describing Nd and Sr isotopic variations in oceanic basalts. We thus have to choose whether to retain the established assumptions and explain the new data as deviations from the rule, or to suspend the assumption of the linear nature of the data and

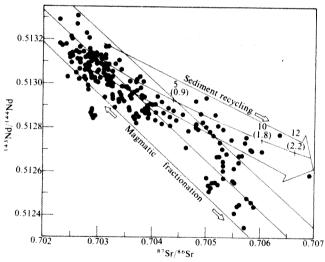


Fig. 3 Variation of ¹⁴³Nd/¹⁴⁴Nd with ⁸⁷Sr/⁸⁶Sr in oceanic basalts. The data clearly define a wedge-shape rather than linear array. One possible explanation is illustrated: magmatic fractionation of Rb/Sr and Sm/Nd (perhaps principally that occurring at mid-ocean-ridges, as suggested in refs 33–35) in time results in a linear band of data while injection of continent-derived sediment into the mantle results in deviation from this linear pattern. Also shown is the effect of mixing a hypothetical ancient oceanic sediment (Rb/Sr and Sm/Nd identical to the deep-sea red clay analysed by McCulloch and Wasserburg³⁷ but having a model age of 2,000 Myr, that is present ⁸⁷Sr/⁸⁶Sr = 0.7239 and ¹⁴³Nd/¹⁴⁴Nd = 0.51204) with a reservoir having the isotopic composition of MORB. This sediment would be roughly appropriate with a 1,800 Myr recycling time for example, refs 35, 36. Ticks and numbers along the mixing line refer to the per cent sediment in the mixture. Numbers in parentheses refer to the case where the depleted end member is mantle with 20 p.p.m. Sr, those without parentheses to mixing with basalt having 120 p.p.m. Sr. Sources of data: Table 1, refs 8, 9, 17, 11, 23, 47–51 and W.M.W (unpublished).

accept the new data into the mantle array without special pleading. If we take the latter course, the mantle array becomes a rather broad band of data where several subsets (from individual island groups) have trends with substantially different slopes. In that case, the assumption of a basically binary system cannot be maintained, and we cannot make any quantitative estimates about a point representing bulk earth. For example, it is quite reasonable to think that the Earth has differentiated to produce six geochemically distinct reservoirs: (1) an upper continental crust; (2) a lower continental crust, (3) an oceanic crust; (4) a depleted mantle region; (5) an undepleted mantle region; and (6) the core. The conventional linear mantle array might then be produced by mixing of (4) and (5), (3) and (4), or perhaps even (1) and (4). The different trends within the mantle array may then be the result of different combinations, but there is no requirement that the bulk earth point must lie within the array. Similar arguments can be made for Pb isotopes, which, on the ²⁰⁷Pb/²⁰⁴Pb versus ²⁰⁶Pb/²⁰⁴Pb diagram, show an overall positive correlation. These can be resolved into several different individual subparallel trends for individual island groups, but virtually all the data lie outside the locus of possible values of bulk earth Pb isotope ratios.

The data from Sao Miguel, Samoa and the Societies are offset from the conventional mantle array in the direction of continental crust and in this sense, the involvement of some kind of crustal component is implied, as Hawesworth et al. 17 suggested with reference to Sao Miguel. If this crustal component is not added during ascent of the magma, then it must be present in the source. The possibility of transport of crustal material into the mantle, suggested by Armstrong²⁵, has recently received wide consideration (see refs 1, 26-36), and Hofmann and White^{34,35} and Chase³⁶ have specifically proposed that recycled oceanic crust is the source of oceanic island basalts. An interpretation of the Sr and Nd isotope data consistent with recycling of oceanic crust is shown in Fig. 3. Magmatic processes operating during the formation of oceanic lithosphere produce correlated variations in Rb/Sr and Sm/Nd. Most of this lithosphere is ultimately returned to the deeper mantle and in time, correlated variations in ⁸⁷Sr/⁸⁶Sr and ¹⁴³Nd/¹⁴⁴Nd result which define the conventional linear mantle array³³⁻³⁵. Addition (and subduction) of continent derived sediment or seawater interaction with crust will, however, produce deviations to the right of the mantle array. The effect of mixing between a hypothetical 'ancient' oceanic sediment and a MORB-type composition is illustrated. As Fig. 3 shows, such mixing can at least qualitatively account for those sources with compositions outside the conventional mantle array and that relatively small amounts of sediment are required. In this interpretation, whether a particular unit of recycled crust has compositions lying outside or inside the conventional mantle array depends on the extent to which the crust has interacted with seawater and/or the extent to which associated sediments are subducted. Sources lying inside the conventional mantle array, such as Tristan and Kerguelen, may represent recycled crust with little or no subducted sediment.

The mixing curve shown in Fig. 3 is illustrative only. The range of isotopic compositions in modern oceanic sediments, much less ancient ones, is not well known; thus any attempt at more quantitative modelling is not justified. It is clear, however, that sediments can be highly variable^{37,38}, both in isotopic composition and in Rb/Sr, Sm/Nd and Sr/Nd ratios (the latter determining the curvature of mixing curves). Even compositions lying inside the conventional mantle array, for example Tristan and Kerguelen, could be explained by mixing between a sediment, for example the North American Shale Composite³⁷, and depleted mantle.

Seawater is also a possible 'continental contaminant', as mentioned above. Sediment has been emphasized here because of the correlated variations in Sr and Nd isotope ratios in the Society Islands and Sao Miguel trends seem to suggest a contaminant containing both Sr and Nd, whereas seawater, which contains essentially no Nd, would be expected to affect only

the Sr isotope ratios. On the other hand, the correlation between Sr and Nd isotope ratios in the Samoan source is weak, which might indicate that seawater is the contaminant.

It is also possible that reservoirs having isotope ratios lying outside the mantle array result from purely magmatic processes. Diagrams illustrating the effects of partial melting on Rb/Sr and Sm/Nd ratios (for example Fig. 5 of ref. 9; Fig. 4 of ref. 17) reveal that these sources could be produced as partial melts of a previously depleted mantle. This possibility is also consistent with recycling of oceanic crust as partial melting of a previously depleted reservoir occurs during MORB genesis.

Models involving recycling of oceanic crust thus provide several possible means of explaining both those data falling outside the conventional mantle array and those sources having ¹⁴³Nd/¹⁴⁴Nd ratios lower than bulk earth. Neither of these features are consistent with binary mixing between a depleted and an undepleted reservoir, such as advocated by Wasserburg and DePaolo³, or with variable depletion of a primordial reservoir4.

Conclusions

In view of the above new data, as well as those of Hawkesworth et al.¹⁷, recycling of oceanic crust into the mantle deserves serious consideration as an important process in the chemical

Received 8 February, accepted 15 March 1982

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evolution of the Earth. However, Pb, Hf, He, Ar as well as Sr and Nd isotopic data, chemical data and geophysical and chemical plausibility must be carefully considered before such models are accepted. The present data support such models rather than confirm them. On the other hand, these new data cannot be explained in terms of simple mixing between an undepleted planetary mantle reservoir and a depleted one, nor can Pb isotope data. Such an undepleted reservoir, though it may exist, must have only a very minor role in present crustmantle interaction. The data are also not explained solely by variable magmatic depletion of an initially homogeneous mantle.

Finally, until the complexities of Sr-Nd isotope systematics in the mantle are better understood, values of 87Sr/86Sr and Rb/Sr for the bulk earth estimated from Sr-Nd relationships in oceanic basalts must be viewed with scepticism.

We thank J. Clark, J. Dymond, R. Duncan, M. Feigenson, M. Lanphere, A. McBirney, W. Melson and H. Banks of the Smithsonian Institution, J. Natland, Z. Peterman, C. Hedge, J-G. Schilling, H. Sigurdsson and S. Sun for providing samples. This work benefited from discussions with, critical reviews by and assistance of M. Tatsumoto, H. Feldmann, C. Göpel, R. Gross, E. Ito, E. Jagoutz, D. James, J. Patchett, Z. Peterman, S. Sun and H. Voshage.

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Kinematics of oceanic thrusting and subduction from basal sections of ophiolites

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The structural analysis of high stress deformation at the base of the peridotite section in many ophiolites in suture zones enables the kinematics of oceanic convergence responsible for the suture to be defined. This deformation results from the thrusting over oceanic crust of a young lithosphere involved in a subduction process. Flow plane, flow line, sense of shear related to the thrusting are geometrically deduced from the study of mineral preferred orientations in the basal peridotite and in the quartzites belonging to the amphibolite sole.

In the palinpsestic reconstruction of palaeotectonics, a key problem is to determine kinematics of plates (direction and sense of relative motion) at destructive margins. At present, the following information is available.

First, the direction of the palaeo suture zone is assumed to be parallel to the trend of ophiolite and blueschist lineaments and to the alignment of eruptive bodies which are ascribed to an active margin magmatism. However, no information is available on the direction of motion of the subducting plate. This direction may be nearly parallel to the suture direction as is the case along the western boundary of the Pacific plate¹ and along the eastern segment of the Hellenic trench system²

Second, the sense of the palaeo subduction is deduced from: (1) the different geological characters between the active and

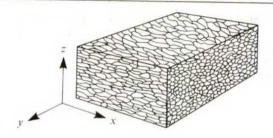


Fig. 1 x, y, z axes of the finite strain defined from the shape fabric: foliation (xy plane) and mineral lineation (x direction).

passive margins of the suture. The active margin is characterized by arc sedimentation, andesitic—granodioritic magmatism, and K/Na polarity, whereas the passive margin is characterized by a sedimentation of continental rise type. Such a simple scheme may be obscured by complex collision situations. (2) The sense of thrusting of the ophiolites which is inferred from their position with regard to the former oceanic crust from which they derived. This criterion is still ambiguous as obduction has been related to either sense of subduction³⁻⁵. Moreover it is not always possible to recognize a thrust whose kinematics is connected to the subduction process from one due to later nappe emplacement, possibly driven by gravity.

A new approach to this problem is now proposed, based on the study of basal tectonites and metamorphic aureoles of many ophiolites. These formations have been ascribed to oceanic thrusts⁶⁻⁸. Kinematic analysis of deformation mainly in the peridotite mylonites makes it possible to deduce the direction and sense of transport of the upthrust lithosphere which may then be related to the direction of convergence of the plates.

Large homogeneous deformations

The kinematic analysis of homogeneous plastic deformation has recently been developed, in particular in peridotites^{9,10} and in quartzites¹¹.

In rocks deformed by homogeneous plastic flow, we accept the concept that the principal axes of the finite strain ellipsoid $x \ge y \ge z$ coincide with the principal axes of the shape anisotropy of minerals, with z perpendicular to the foliation and x parallel to the mineral lineation (Fig. 1). In peridotites, the principal directions of strain are marked by the shape of the olivine crystals, or better, in the field, by spinel or feldspar aggregates. In quartzites they are marked by the shape of quartz crystals, often emphasized by phyllitic bands.

Fabric measurements in peridotites and quartzites show that the mean orientation of slip directions and slip planes in the principal minerals (olivine and quartz respectively) are related to the axes (x, y, z) (Fig. 1) of the finite strain ellipsoid so that the mean direction of slip commonly lies at small angle to the x axis (Fig. 2a). This obliquity has been observed in numerous cases and in various materials; it has been attributed to shear flow (see refs 10, 12), with the inference that the flow plane and the flow line can be equated with the mean orientation of the slip planes and the slip lines respectively in the principal rock-forming minerals (Fig. 2b). The sense of shear flow is deduced from the sense of the obliquity. This interpretation has been confirmed by planar simulation of simple shear 13 , by studies in natural shear zones where the flow geometry is well known $^{14-16}$ and recently by experimental deformation in ice 17 .

The obliquity of the kinematic directions relative to the x and z axes of the finite strain ellipsoid can be deduced from petrofabric measurements (Fig. 2b), or more simply from thin sections cut parallel to the xz plane observed between crossed polarizers (Fig. 2a).

Oceanic thrusting conditions

The basal peridotites in many ophiolite complexes (see refs 7, 18) overlie a sole of metamorphic rocks with facies grading downwards from granulite and/or amphibolite facies to greenschist facies. This metamorphism is developed in oceanic crust formations as a result of mechanical and thermal effect of an overriding slice of oceanic lithosphere 6.7.19. Thirty ophiolite

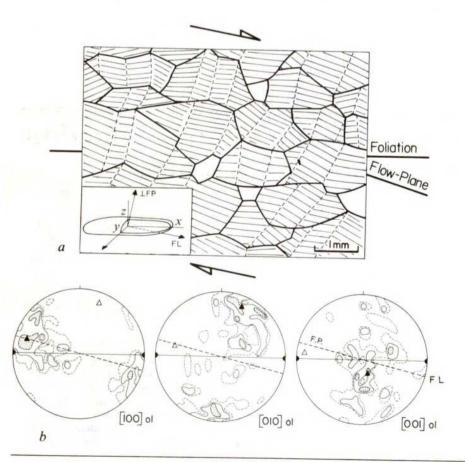


Fig. 2 Schematic section in the xz plane of an olivine or quartz aggregate. Dashed lines are the traces of sub-boundaries (visible under crossed polarizers), perpendicular to the slip direction. Solid lines are the traces of slip planes (not directly visible under crossed polarizers). The sense of shear is deduced from the orientation of foliation relative to average slip plane (equated to the flow plane, FP) and/or lineation relative to average slip direction (equated to the flow line, FL). b, Equal area projection (lower hemisphere) of the olivine crystallographic axes. The flow plane (FP) (dashed line) is equated with the average slip plane (010)ol, the flow line (FL), with the average slip direction 100_{ol}. Solid line, foliation; ● mineral lineation; A, computed maxima and minima of crystallographic directions.

massifs are known which present such a metamorphic sole¹⁸. The following description relies on structural studies conducted in six massifs and especially in the Bay of Island (Newfoundland)²⁰ and Samail (Oman)²¹.

Above the contact with the amphibolites and within a few hundred metres of it, the peridotites of the ophiolite suite

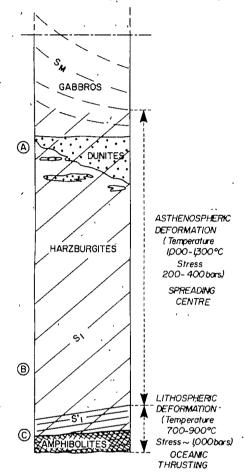


Fig. 3 a, Schematic cross-section through the lower part of an ophiolite sequence showing structures of the lithospheric deformation (S_1') superimposed on that of the asthenospheric deformation (S_1) . S_M is the cumulate layering. A, hypersolidus asthenospheric deformation; B, subsolidus asthenospheric deformation; C, lithospheric deformation. These zones refer to the models in Fig. 5.

exhibit mylonitic textures indicative of large strain-high stress deformation (Fig. 3). According to the olivine neoblast size piezometry, the stress is evaluated at 1-2 kbar (ref. 18). The temperature in these peridotites is estimated at 700-900 °C if they are considered to be in thermal equilibrium with the underlying amphibolites and granulites. This deformation is clearly distinct from the high temperature-low stress one (Fig. 3) which affected the whole ultramafic sequence and originated from the asthenospheric flow in a spreading environment. Temperatures during the asthenospheric flow are close to the peridotite solidus (1,250 °C) and stress is of the order of 200-300 bar (ref. 18).

The amphibolites underlying the peridotites often contain intensely deformed quartzite bands deriving from the silica-rich oceanic sediments. These rocks may have recorded several superimposed tectonic events corresponding to decreasing temperatures²². The highest temperature deformation, contemporaneous with the contact metamorphism, is ascribed to the oceanic thrusting. It produces a penetrative foliation and a lineation which are parallel with those in the overlying peridotites. The kinematic analysis of the thrusting can equally well be undertaken in the mylonitic peridotites or in the quartzite bands from the amphibolites. However, the low temperature limit for plastic deformation of quartz may mean that quartzites also recorded later events such as nappe emplacement.

Kinematics of oceanic thrusting

The proposed method has already been applied in the study of the Bay of Island ophiolite in Newfoundland²⁰ and of the Samail ophiolite in Oman²¹.

Figure 4 illustrates the case of the case of the Samail ophiolite. The high stress deformation traced at the very base of the peridotite has the same geometry as the deformation recorded in the amphibolites; it contrasts with the low stress—high temperature deformation producing coarse porphyroclastic textures in the main part of the peridotite sequence. Lattice preferred orientations of orthopyroxene (Fig. 4a) in mylonitic peridotite and of quartz (Fig. 4b) in quartzites display an obliquity to the penetrative foliation and lineation which indicate the same sense of shear for both rock types.

In geodynamic situations, two possible decoupling surfaces are envisaged (Fig. 5), in which thrusting of young oceanic crust could be initiated, due to oceanic convergence.

First, at a spreading axis itself²³, if there is a shift from oceanic expansion to convergence. The compressive force may initiate thrusting along the lithosphere—asthenosphere interface (Fig. 5a). The decoupling surface would correspond to the isotherm representing the lower limit for peridotite high temperature

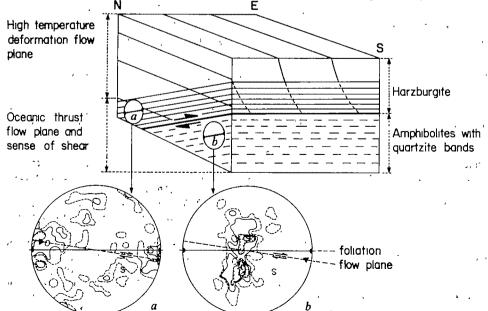


Fig. 4 Relationship between deformation in the amphibolites of the metamorphic sole and in the basal harzburgites in the Samail ophiolite (Oman). a and b are equal area lower hemisphere projections of crystallographic axes; 100 measurements; xz finite strain reference system (S = geographical south); solid line parallel to the lineation and trace of the foliation; the flow plane is deduced from fabric data; double arrow = sense. of shear. a, [001] orthopyroxeneperidotite slip direction in mylonite: the ▲. computed maximum (flow direction). b, c axis [0001] quartz in quartzite: A, the computed pole of the girdle (flow direction).

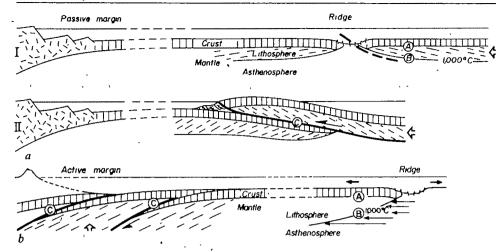


Fig. 5 Possible geodynamic locations of oceanic thrusting: a, at a spreading axis, along the 1,000 °C lithosphere/asthenosisograde. phere interface. A flat isograde corresponding to fast spreading will favour the expected situation: b, in a subduction environment at an active margin, at the time when new created crust is involved in the subduction process. A, B, C indicate zones where asthenospheric and lithospheric deformations are imprinted in the peridotite; it refers to level A, B, C, in the ophiolite sequence in Fig. 3.

plastic flow (>1,000 °C). Depending on the spreading rate, the isotherms are variously inclined (10°-25° for spreading rates 5-1 cm yr⁻¹). Such angles compare favourably with those proposed for basement-thrust24

Second, at an active margin, where downward bending of the lithosphere plate develops elastic deviatoric stresses, seaward of the trench8. In the case of a young lithosphere, the

Received 19 January, accepted 25 February 1982

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elastic stresses are located at shallow depth along a roughly horizontal decoupling surface.

In the first case, the thrusting will predate the establishment of a subduction process, as it is related with the initiation of the convergence process. In the second case the thrusting may postdate an active subduction, occurring at the time when young crust is involved in the subduction process.

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Enzymatic synthesis of bacteriophage fd viral DNA

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An enzyme system with requirements similar to those for replication of phage fd replicative form (RF) DNA in bacteriophage fd-infected cells has been reconstituted with purified fd gene 2 protein, and DNA polymerase III holoenzyme, DNA binding protein I and rep-protein (rep-helicase) of Escherichia coli. The system generates viral circular single strands, which are infective for E. coli spheroplasts. Parental and newly synthesized DNA are covalently connected in early stages of replication, as expected for DNA replication using the rolling circle mechanism. Single-stranded tails of the rolling circle intermediates are cleaved after a full round of replication by gene 2 protein and circularized by the same enzyme molecule.

THE F-pili specific filamentous bacteriophage fd, which is closely related to phages M13 and f1, has been extremely useful in the study of structural and functional aspects of small viral DNA genomes. The organization of the circular fd genome and its nucleotide sequence have been analysed in detail, and the single-stranded character of fd DNA has given rise to the construction of unique vector systems for cloning and quick sequencing of genes1.

The life cycle of bacteriophage fd² begins with the entry of the viral DNA into an Escherichia coli cell, where it is immediately converted into a double-stranded replicative form. RF replication then occurs, forming a pool of double-stranded DNA molecules. RF replication requires the phage-encoded gene 2 protein as the sole enzyme function encoded by the phage³. In a late stage of infection the pool of RF molecules

is used as a matrix for the production of new viral single strands.

On the basis of genetic data, host functions of Escherichia coli essential for replication of filamentous phages have been assigned to DNA polymerase III and I4, RNA polymerase, DNA gyrase and probably DNA binding protein I⁵. The involvement of E. coli RNA polymerase and DNA gyrase has been deduced from the inhibition of phage replication by the drugs rifampicin⁶ and nalidixic acid⁷. Rifampicin specifically interferes with the synthesis of the complementary strand in the stage of duplex propagation8, whereas nalidixic acid affects RF replication, but not the conversion of single strands to RF⁷. Another E. coli gene function involved in RF replication of small bacteriophages is encoded by the rep gene⁹. Participation of the dnaB and dnaG gene products has also been proposed for replication of filamentous phages^{10,11}, although their requirement in enzymatic replication of fd RF has not been demonstrated.

Enzymatic studies have revealed the participation of the *E. coli* proteins DNA binding protein I, RNA polymerase, DNA polymerase III holoenzyme, DNA polymerase I and DNA ligase in the conversion of single strands to RF¹². The polymerases are required, respectively, for synthesis of the RNA primer, elongation of the DNA chain and excision of the primer concomitant with gap filling¹³. The DNA binding protein directs the initiation event and supports DNA polymerase III in DNA replication. DNA ligase seals the double-stranded DNA into the relaxed circular covalently closed form (RFIV).

To initiate double-strand replication, the relaxed RF is converted into supercoiled RFI by DNA gyrase. Phage fd gene 2

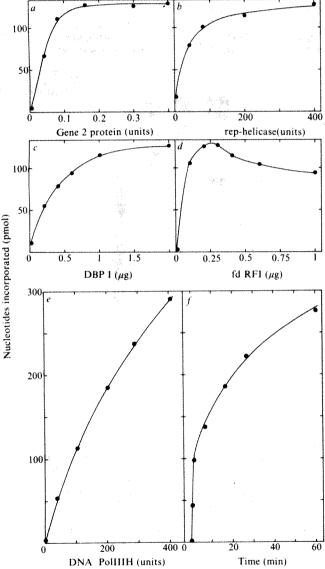


Fig. 1 Proteins required for enzymatic replication of fd RF. The standard incubation mixture contained 200 ng fd RFI (600 pmol of nucleotides), 0.08 U of fd gene 2 protein, 100 U of E. coli rep-helicase, 1 μg E. coli DNA binding protein I, 100 U of E. coli DNA polymerase III holoenzyme in 20 μl of 20% sorbitol, 50 mM HEPES (pH 7.5), 0.1 mg ml⁻¹ bovine serum albumin, 5 mM 2-mercaptoethanol, 5 mM MgCl₂, 500 μM ATP, 50 μM of dATP, dGTP and dTTP, and 25 μM of ³H- or ³²P-labelled dCTP (1,000–3,000 c.p.m. pmol⁻¹). Incubation was for 5 min at 30 °C. The nucleotide incorporation was stopped with 5% perchloric acid and the acid-insoluble radioactivity determined. The sources of the proteins have been described elsewhere ¹⁷. a, fd gene 2 protein; b, E. coli rep-helicase; c, E. coli DNA binding protein I; d, phage fd RFI; e, E. coli DNA polymerase III holoenzyme. f Gives the time course of nucleotide incorporation.

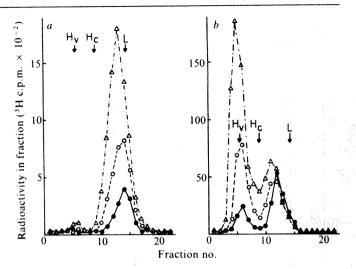


Fig. 2 Analysis of bromouracil-labelled replication products in alkaline caesium sulphate. In the standard incubation mixture, dTTP was replaced by bromodeoxyuridine triphosphate. Incubation was stopped at various times by the addition of 80 μl of 0.1 M NaOH and 10 mM EDTA. The Cs₂SO₄ gradients were run in a Beckman 50 Ti fixed-angle rotor at 40,000 r.p.m. and at a mean density of 1.46 g ml⁻¹ for 28 h at 20 °C in 0.08 M NaOH/10 mM EDTA. Increasing density is from right to left. Panel a: ●, 15 s; ○, 30 s; △, 60 s. Panel b: ●, 2 min; ○, 4 min; △, 8 min of incorporation. External markers were bromodeoxyuridine-substituted fd viral strands (H_v) and complementary strands (H_c) and fd single-stranded DNA of light density (L).

protein cleaves this DNA in the viral strand¹⁴ at a specific site^{15,16}. Gene 2 protein supports strand unwinding by rephelicase (rep-protein)¹⁷ and the 3'-hydroxy end of the viral strand is extended by DNA polymerase III holoenzyme. Both reactions require the presence of E. coli DNA binding protein I. Cleavage and circularization of replicated viral strands by fd gene 2 protein have been demonstrated in model systems^{18,19}. In addition, replication of duplex DNA from filamentous phages has been reported for a subcellular E. coli system²⁰.

Information on the mechanism of DNA replication has been obtained with Φ X174 DNA for the conversion of viral strands into supercoiled duplex DNA²¹, and its simultaneous replication into viral and complementary strands²². Although this system is structurally related to the phage fd replication system, it differs substantially regarding enzymatic requirements and the mode of action of the enzymes involved^{13,23}. Replication systems using linear double-stranded DNA as a substrate, that is, the T4²⁴ and the T7²⁵ in vitro systems, have given insight into the mechanistic principles of a replication fork.

Here, we analyse an enzyme system for replication of doublestranded fd DNA which is assembled with proteins present in the fd-infected cell. We will discuss in detail the mode of action of gene 2 protein as the key enzyme in the production of circular bacteriophage fd viral strands.

Enzymatic requirements for replication of fd RFI

In accordance with the requirements for phage fd replication in vivo, an enzymatic system was set up for components stimulating DNA synthesis on supercoiled fd RFI DNA. The influence of the individual proteins—fd gene 2 protein, E. coli rep-helicase, E. coli DNA binding protein I and E. coli DNA polymerase III holoenzyme—and of the template DNA fd RFI on the incorporation of deoxyribonucleotides is shown in Fig. 1. The DNA and the replication proteins can be added up to saturating amounts in standard incubation conditions, except that increasing amounts of DNA polymerase III holoenzyme do not reach a plateau of nucleotide incorporation (Fig. 1e). DNA synthesis starts immediately after incubating the assay mixture at 30 °C, decreases slightly after 5 min of incubation,

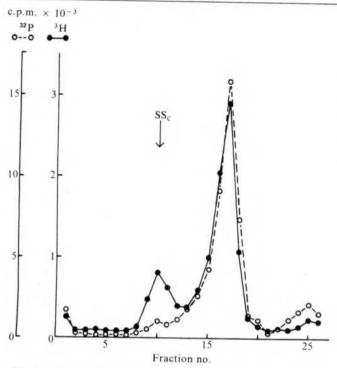


Fig. 3 Gradient analysis of replication products. a, Sedimentation in neutral sucrose. The template $^3\text{H-fd}$ RFI (10,000 c.p.m.,300 ng) was isolated from phage-infected cells. A volume of $60\,\mu\text{l}$ (three times the assay system described in Fig. 1 legend) with label in $[\alpha^{-3^2}\text{P}]\text{dCTP}$ $(1,700\text{ c.p.m. pmol}^{-1})$ was incubated for 5 min at 30 °C. After the reaction had been stopped with NaCl/EDTA, the sample was diluted with $100\,\mu\text{l}$ of 1 M NaCl in Tris-HCl/EDTA and layered on top of a neutral sucrose step gradient. Sedimentation was performed in a Beckman SW60 Ti rotor for 2.5 h at 50,000 r.p.m. and $20\,^{\circ}\text{C}$. To fractionate the gradient the tubes were punctured in the bottom. Sedimentation is from right to left. The position of fd circular single strands (SS_C) as external marker is indicated.

and is still occurring at $60 \, \text{min}$ (Fig. 1f). At that time, the amount of nucleotides incorporated approaches the equivalent of one round of viral strand synthesized per RF molecule.

Products generated in the replication system

To determine the mode of replication implemented in the enzyme system, density label was incorporated into newly synthesized DNA via substitution of thymidine triphosphate by bromodeoxyuridine triphosphate. Subsequent equilibrium sedimentation analysis of the replicated DNA in denaturing conditions reveals label in two distinct positions: at heavy density, corresponding to heavy viral marker DNA, and at intermediate-light density (Fig. 2). The latter material, which is predominantly found at early incubation times, indicates a covalent connection of heavy newly synthesized and light parental DNA. These results support the rolling circle model for fd double-strand replication which implies synthesis of the viral strand by covalent elongation of the parental viral DNA. The tail of single-stranded DNA generated in this reaction is then cleaved off late in the replicative cycle. Heavy material in the density gradient represents this kind of processed viral DNA which is found after ~60 s of incubation.

To monitor the production of fd single strands, ³H-labelled fd RFI was used as template and newly synthesized material was labelled with ³²P-dCTP. Sedimentation of the products in neutral sucrose separated single-stranded from double-stranded DNA (Fig. 3). Quantitative evaluation of the distribution of both labels revealed that 25% of the total radioactivity of the template occurred as single-stranded DNA. About 6% of the newly synthesized ³²P label sedimented in the position of single-stranded material. These results show that half of the template molecules were replicated and the single strands with

parental label were cleaved from the double-stranded core, but label from the second round of replication could not be detected efficiently by sedimentation in neutral sucrose.

We have further investigated the replication products by electrophoresis in agarose gels, where linear and circular single strands can be separated from each other. Figure 4a shows that the single-stranded newly synthesized DNA migrated in the position of circles, whereas linear single strands of unit length were less than 1% of the circular DNA. This result illustrates the efficient circularization of single-stranded DNA by gene 2 protein in the E. coli replication system. Because no label is incorporated into single strands in the first round of replication, these circles are derived from following rounds of viral strand synthesis.

The production of circular fd single strands was shown by migration with regard to single-stranded marker DNA (Fig. 4a), but also proven by conversion of the material into double-stranded DNA (Fig. 4b, c). The enzymatically synthesized single-stranded DNA was nonspecifically primed with plasmidencoded DNA primase²⁶, the RNA primer was extended with

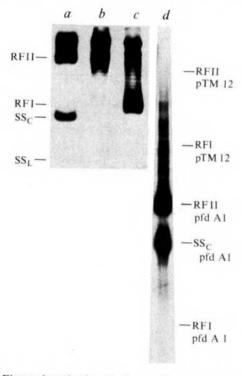


Fig. 4 Electrophoresis of replication products on agarose. a, A standard incubation mixture (see Fig. 1) with $[\alpha$ -(720 c.p.m. pmol-1) was stopped with SDS buffer and the DNA separated on a 1% agarose gel. b, Incubation was as in a but without label. The mixture was then heated to 65 °C for 5 min and incubated after addition of $[\alpha^{-32}P]$ dCTP with DNA primase encoded by the I-like plasmid R64drd11 (from E. Lanka) coli DNA polymerase I (0.1 µg) and T4 DNA ligase (2 µg)³ for 1 h at 20 °C. c, The products of b were incubated with 2 units of purified DNA gyrase 17 for 30 min at 30 °C. External markers were single-stranded circular fd DNA (SSC) isolated from the purified phage, linear single-stranded fd DNA (SSL) obtained by cleavage of fd single-stranded circles with pancreatic DNase; fd RFI (RFI) isolated from fd-infected cells; fd RFIIo (RFII) generated by cleavage of RFI with fd gene 2 protein and linear single strands (RFIII), obtained by cleavage of fd RFI with gene 2 protein in the presence of Mn²⁺ (ref. 14). d, Replication of plasmid pfdA1 (2.4 kb)³. The plasmid (200 ng), which contains a cloned 261-base pair origin fragment of phage fd, was replicated in conditions described in Fig. 1 legend. In addition, 50 ng of the pBR derivative pTM12 (6.2 kb)³, bearing the gene 2 of fd but not the fd origin, were present in the assay and served as an internal control for the specificity of the replication system. The products were separated by electrophoresis through 1.5% agarose. The singlestranded nature of the pfdA1 replication product, SSc, was controlled by digestion with S1 nuclease.

Table 1 Gene 2 protein-mediated activation of fd RF replication

	DNA syr the four-c system	omponent	Product of first
Substrate	-G2P	+G2P	round of replication
fd RFI	5	120	SS circles
fd RFIV	6	3	444444
fd RFII _o	18	· 84	SS circles
fd RFII	14	103	SS linear DNA of unit length
fd RFII	16	15	anaman .

The template DNA (200 µg) was added to the standard assay system (see Fig. 1). Products were analysed by agarose gel electrophoresis and subsequent quantification of unlabelled (first round of replication) and labelled (following rounds of replication) single-stranded (SS) DNA species. The analysis was accompanied by electron microscopic evaluation 17. G2P, gene 2 protein; fd RFII_o, phage fd replicative form DNA specifically cleaved with fd gene 2 protein; RFII_p represents RFII_o without 5'-phosphate on the cleaved viral strand.

DNA polymerase I and the strands were finally sealed with T4 DNA ligase (Fig. 4b). Subsequent treatment of this DNA with gyrase should shift all closed circular molecules into the position of supercoils in the gel. This indeed can be observed in Fig. 4c, proving the circular nature of the originally synthesized single strands. Control experiments in the absence of primase showed that an excess of T4 DNA ligase prevented incorporation of label into double-stranded fd RFII, which was also produced in the replication system.

Enzymatic replication with DNA species different from fd RFI

The initiating cleavage reaction conducted by gene 2 protein is limited on supercoiled DNA substrates which harbour the replication origin of phage fd¹⁴. The small 2.4-kilobase (kb) plasmid pfdA1, which contains a cloned fragment of the fd origin³, should fulfill these properties. Figure 4d shows that the described in vitro system uses the 261-base pair origin fragment and replicates the plasmid with high efficiency, producing single-stranded DNA.

Previous studies on the mechanism of gene 2 protein revealed a specific reversible binding affinity of the enzyme to its cleavage site in fd RFII_o. This weak interaction is an essential prerequisite for unwinding of fd RFII_o by rep-helicase and DNA binding protein I¹⁷. Consequently, fd RFII_o is a suitable substrate in the replication system for production of circular single strands like fd RFI, if gene 2 protein is present (Table 1). Removal of the 5'-phosphate group in fd RFII_o still produces a substrate (fd RFII_p) which is efficiently propagated by gene 2 protein and the E. coll proteins. However, linear rather than circular single strands are generated in this case due to a missing phosphate group required for ring closure in the first round of replication. Relaxed RFIV, and fd RFII with a nick outside the replication origin, were unsuitable templates in the replication system composed of the four proteins (Table 1).

Biological activity of the replicated single-stranded circles

Faithful DNA replication should produce DNA with the informational content of the template DNA; imprecise circularization of viral single strands should result in a loss of their biological activity. We analysed the *in vitro* replication products in an $E.\ coli$ protoplast system in which double-stranded phage DNA is ~100 times less infective than single-stranded circular DNA (Table 2).

The replication products from a standard incubation assay were deproteinized, because *E. coli* DNA binding protein I interferes with the infectivity of single strands²⁷. Plaque formation in the spheroplast assay (Table 2) showed infective centres

after an enzymatic replication of the DNA, which exceeded infectivity of double-stranded fd RF by two orders of magnitude. Single strands produced by strand unwinding of RF increased this background, but linear viral strands or circular complementary strands were apparently less infective than circular viral strands, as already found for phage Φ X174 DNA²⁸. These results demonstrate effective fd viral strand replication in our system and the precise circularization of the synthesized viral strands by gene 2 protein.

The role of fd gene 2 protein in phage duplex DNA replication

Replication of the viral strand of fd RF is initiated by the phage-encoded gene 2 protein^{29,30}, which introduces a specific single-strand break into the viral strand of the phage RF. The cleavage site of gene 2 protein has been localized in a region of palindromic symmetry¹⁵. This finding and the observation that gene 2 protein cuts both strands of fd RF in the presence of Mn²⁺ (ref. 14) suggest that in physiological conditions two molecules of the enzyme bind to the cleavage region, but only one reacts with the viral strand. Consequently, dimer formation at the cleavage site is believed to be essential in the initial step, and also for processing of rolling circle structures (see below). Concomitant with the cleavage of the viral strand, gene 2 protein may attach transiently to one of the termini of this strand by forming a covalent bond. This intermediate may provide the energy to seal the previously opened strand in a topoisomerase-like manner¹⁴. The attachment of the enzyme to the nicked DNA strand is either followed by the formation of RFIV or the hypothesized protein–DNA complex is

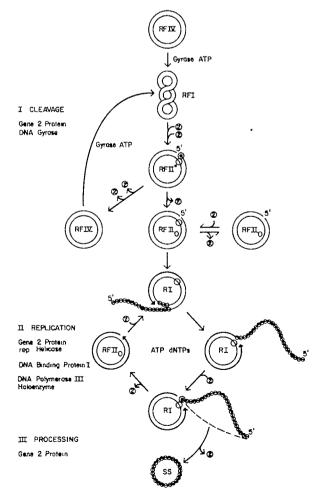


Fig. 5 A model for enzymatic replication of phage fd RF and the involvement of bacteriophage fd gene 2 protein.

Table 2 Infectivity of newly formed fd single strands after enzymatic replication of phage fd RF

• • • • • • • • • • • • • • • • • • • •	
DNA source	Infective centres per 1×10 ¹⁰ molecules of template DNA
Replication of fd RF	2,780
Enzymatic unwinding of fd RF*	400
Cleavage of fd RFI by fd gene 2 protein†	5
fd RFI‡ ,	47
fd viral single strands	8,500

Phage fd RF was replicated for 20 min as described in Fig. 1 legend. The incubation was stopped after 5 min at 30 °C by the addition of EDTA. 0.05 M Tris-HCl (pH 8.0) was added to a final volume of 0.3 ml and the sample was treated with phenol. Phenol in the aqueous phase was removed by extraction with ether. The sample was then mixed with 0.2 ml of a protoplast suspension of cells from E. coli K-12 strain W6 (ref. 33). After 15 min at 37 °C the protoplasts were mixed with the indicator strain 1101 and plated on PAM agar.

* Unwinding with fd gene 2 protein, rep-helicase and DNA binding

protein I. The products are circular complementary strands and linear viral strands.

† The reaction with gene 2 protein gives RFIV and RFII. (ref. 14).

‡ Transfection rate variable.

rearranged to become active for initiation of the unwinding of fd RFII.

Analysis of the cleavage site in fd RFII has revealed free 3'-hydroxy and 5'-phosphate ends which are substrates for ligation with DNA ligase or for nick-translation with DNA polymerase I^{14,15,31}. However, the complementary strand at the cleavage site seems to be preferentially protected against endonucleolytic digestion with Bal31 nuclease, in contrast to randomly located nicks¹⁷. It has been shown that this protection is due to a reversible binding of gene 2 protein to the cleavage site, which is necessary for specific initiation of the unwinding and the replication reactions. RFIV which is produced in the initiation reaction in addition to fd RFII, needs to be recycled into RFI by DNA gyrase before being processed once more by gene 2 protein (Fig. 5).

The subsequent replication process, outlined in Fig. 5, requires the E. coli proteins DNA polymerase III holoenzyme, rep-helicase and DNA binding protein in addition to the fd gene 2 protein. The rep-helicase accomplishes unwinding of fd RFII in collaboration with DNA binding protein I17. DNA polymerase III holoenzyme extends the 3'-hydroxy end released by the cleavage reaction and proceeds in a rolling circle-type replication. As deduced from experiments carried out in the phage T4 in vitro replication system¹⁹, one molecule of gene 2 protein remains bound to the complementary strand of fd RF during replication. As replication ceases near the origin in the presence of gene 2 protein, this enzyme may serve as a signal for termination after a complete round of replication. A second molecule of gene 2 protein is then required to cut off the replicated single strand, based on the difference in the kinetics of both steps19. The parental RF molecule is released in an open form and therefore immediately used as a template Received 10 December 1981, accepted 1 March 1982

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for further replication cycles. For late infection with filamentous phages the occurrence of pulse label in the viral strand of RFIV³² may be due to conversion of RFII molecules into doubly closed DNA by DNA polymerase I and DNA ligase.

The unwound single strands are readily covered by DNA binding protein as replication proceeds¹⁷. This stoichiometric function can be substituted by phage-encoded DNA binding protein (fd gene 5 protein), although catalytic amounts of DNA binding protein I remain essential for unwinding and synthesis of DNA (unpublished results).

In a final reaction, the unwound viral single strand is separated from the parental RF molecule and circularized (Fig. 5). The function of gene 2 protein is solely responsible for this process, as the E. coli proteins in the replication system can be substituted completely by T7 enzymes, that is, T7 DNA polymerase and T7 DNA primase (gene 4 protein)18. However, as we demonstrated by use of the T4 replication system, processing only occurs during concomitant DNA synthesis¹⁹. In the absence of enzymes providing DNA synthesis, long-tailed rolling circle structures are not processed per se, which is consistent with the observation that circular viral single strands are not accepted as a substrate by gene 2 protein¹⁴. We therefore believe that, as for cleavage of fd RFI, a defined DNA conformation in the cleavage region is required for processing the rolling circle intermediate. This situation may be realized by a partially melted double-stranded structure caused by the superhelical tension in fd RFI or due to unwinding, when the replication fork enters the cleavage region of the rolling circle intermediate19

The circularization of phage fd DNA by gene 2 protein basically resembles the sequence-specific topoisomerase reaction of this enzyme on fd RFI. Although we cannot demonstrate the independence on ATP of this reaction, because strand unwinding requires ATP, we have shown previously that the nicking-closing reaction of gene 2 protein on fd RF is unaffected by high-energy compounds like ATP14. As cleavage of singlestranded tails from the rolling circle intermediate occurs regardless of the composition of the 5' end of the tail¹⁹ (Table 1), we conclude that gene 2 protein attaches to the 3'-hydroxy end so as to conserve the cleavage energy for circularization of this DNA. Some affinity of the 5' end at the tail of the rolling circle for the gene 2 protein molecule on the complementary strand may favour the replicated viral strand looping back to the double-strand core, thus facilitating viral strand circularization after a round of replication. Circularization may also be supported by pairing palindromic sequences at the replication origin.

If the 5'-terminal phosphate group of the cleaved viral strand is missing, no circle can be formed (Table 1)17 and gene 2 protein dissociates from the DNA. Thus, gene 2 protein can act in two different ways: either it uses the conserved chemical energy to form a new phosphodiester bond, or, as for the production of RFII_{or} it leads to a decay of the protein-DNA complex, which then simply mimics an endonucleolytic reaction.

We thank Erich Lanka for the donation of DNA primase, Marlies Kaiser, Erna Piaskowski and Ilge Wilhelm for technical assistance and Hermann Bujard, David H. Figurski and Robb Moses for comments on the manuscript.

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LETTERS TO NATURE

Transient emission of ultra-high energy pulsed γ rays from Crab pulsar PSR0531

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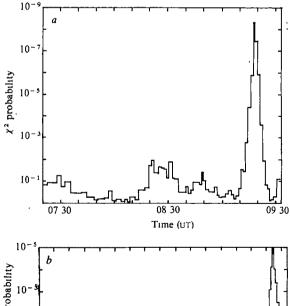
A new experiment has been established to measure the energy spectrum of ultra-high energy (UHE) γ rays (E > 2,000 GeV) from a number of celestial objects. During the commissioning of the equipment several potential sources were studied, including the Crab pulsar PSR0531. UHE γ rays from this object have been observed in previous experiments to be practically all pulsed1-4, but no single observation has been of high statistical significance, and the flux values have been difficult to reproduce⁵. The current observations are based on 34 h of exposure between 25 September and 2 November 1981, and show overall an integral pulsed flux in agreement with our estimate of the average of previous data; most important, they also show strong evidence for a burst of pulsed emission of ~15 min duration. There had been previous suggestions that the Crab pulsar is variable at the energy we used and at slightly lower energies^{1,3}, but at time scales of months or days. The present work is the first to demonstrate short duration (15 min) emission and to offer an explanation of previous apparently discordant results.

The details of the experiment are given elsewhere⁶; it comprises an array of four independent telescopes designed to detect the weak optical Cherenkov emission from electron showers produced in the atmosphere by cosmic γ rays or cosmic-ray particles. It is sited at an altitude of 1,451 m in the Great Salt lake desert, USA, Each telescope contains three paraxial mirrors of 1.5 m diameter focusing the Cherenkov light onto photomultipliers with a field of view of 1.7° FWHM. The array is designed to enable sources to be continuously tracked, with the arrival directions of cosmic γ rays or particles entering the field of view being measured by using the relative time of arrival of the Cherenkov light front at each telescope. When searching for point sources of continuous emission, the detectors are operated with procedures designed to stabilize the threshold energy⁶, which results in an elevated threshold and consequently a lower shower detection rate.

The results reported here are based principally on the universal time (UT) of arrival of cosmic-ray events in the field of view. The search for periodic emission requires the highest possible rate of shower detection, and so the stability procedures were not employed, in order to reduce the threshold energy. The times of arrival of UHE γ rays and the much more numerous background cosmic-ray particles were recorded with a resolution of 1 µs, and with an absolute uncertainty in UT of ~1 ms. After reduction to the Solar System's barycentre, the times were unfolded using an ephemeris derived from contemporary radio observations (A. G. Lyne, personal communication) (period = 0.033263833076 s, derivative = 36.416 ns day⁻¹, epoch = 2444910.5). The phase histograms so produced were searched for evidence of non-uniformity, arising from pulsed γ rays being detected at a uniform rate over the period of observation. Overall, no significant signal was found.

Ideally, periodicity should be searched for by correlating the observed γ -ray arrival time series with the expected light curve, using a likelihood ratio method. This cannot, however, be used in this case because there are no well measured data on the

light curve at these energies. There are only estinates based on measurements at lower energies, to which a triori probabilities cannot be assigned. The usual procedure then adopted is to invoke Poincare's Theorem—that a random tine series of sufficient length, unfolded with an arbitrary period results in a uniform phase distribution. A test for the unifornity of the phase distribution, resulting from the experimental time series being unfolded with a trial period, is taken to inlicate the absence of that period in the time series. Previous searches for periodicity have usually used a binned phase distribution and then either Pearson's χ^2 test for uniformity, or have searched by eye for evidence of an excess in one or more bins, with an allowance for the extra degrees of freedom (d.f.) ariling from the arbitrary choices. These methods suffer from sone disadvantages due to the binning and the arbitrary choices to be made. However, more importantly, none of these constitutes the most powerful test, even if independent tests are combined. The 'uniformly most powerful' test for the uniformity of a circular (phase) distribution, which also avoids the problem of arbitrary choice, is the Rayleigh test⁷ in which the resultant vector, Φ, formed from unit vectors at the phases of the events in the time series is tested for significance. The statistic $2N\Phi^2$ (where N is the number of events in the time series) is distributed as a χ^2 distribution with 2 d.f. This is equivalent to the test for the significance of an amplitude in power spectral analysis, in which the amplitude of any period from a noise series also has a χ^2 distribution with 2 d.f. (ref. 8). This procedure has been extensively checked by testing randomly generated time series, both with and without an added sampled periodic component. The distribution of the resultant, ϕ , was found to conform closely to that predicted in the case of such noise series, and for randomly chosen periods in the experimental series. It has been possible to verify the distribution only down to a probability of 10⁻⁵ due to the limitations of the



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Fig. 1 The probability of lack of periodicity at the radio period for data in 15 min intervals during observations on a, 23 October 1981, b, 31 October 1981.

pseudo-raniom number generator used, and to the finite length of our experimental data. An added periodic component was always detected at the injected strength but with a sampling uncertainlygiven by the above distribution.

Random sampling of a noise series incorporating a true periodicityshould give, and in our simulations gave, an effect close to the nearest Fourier harmonic of the duration of the observations, deviating from the actual period due to the effects of sampling. To check for periodicities appearing in only part of our data, for errors in the application of the ephemeris or the Solar system barycentre corrections, and for deviations of the Crab pulsar from the ephemeris, periodicity was searched for by sveeping around the pulsar's expected period. The counting ate of the array varies with time due to the changing zenith angle of the source, giving rise to a further spreading of the samping range for a periodicity. Starting at 09.14 UT on 23 October, a strong effect was noticed at the directly adjacent Fourier larmonic to the ephemeris period (the harmonic separation beng based on the total duration of the observation). The charce probability using the Rayleigh test was 10⁻⁴ with 30 d.f., which was felt to be an indicator of pulsed activity; to become consistent with the ephemeris period (that is, at the same Fourier harmonic), the duration of the pulsed emission was required to be shorter than the duration of the observation.

Each night's data were therefore split into 15 min sections, the length being dictated by that required to make the effect seen or 23 October occur in the same Fourier harmonic as the ephemeris period. The sections were each unfolded with the appropriate barycentric period, and the probability of uniformity calculated. Using this procedure the number of d.f had been expanded from 1, in the case of the whole 34 h treated as one set, to 130 d.f. Nevertheless, the chance probability for one of the sections taken on 23 October decreased to $< 10^{-6}$. On 3. October another section showed possible activity with a chance probability of $\sim 10^{-4}$.

To refine the positions of the apparent bursts of periodic emission and also to verify the derived probability values by observing the 'noise' spectrum, a section of fixed length moving in increments of ~ 2 min was used. The result of this procedure is a profile of a data set giving the probability of lack of periodicity (for 1 d.f.) as a function of time with a characteristic duration of 15 min. The profiles of the sets for two nights, 23 and 31 October, are shown in Fig. 1. The total number of d.f. has now expanded to $\sim 1,000$ because of the overlapping of the sections. The peak near the end of the run on 23 October has a Rayleigh test χ^2 value of 37.2 for 2 (internal) d.f., which has a chance probability of 4×10^{-9} . At this peak, $34\pm 5\%$ of the recorded events are periodic, corresponding to an integral flux of $(2\pm 0.3)\times 10^{-10}$ cm⁻² s⁻¹ for the 15 min burst. The threshold energy depends on the zenith angle at which the data are taken and for this observation was 3,000 GeV.

The records of showers arriving during the bursts have been examined for evidence of any properties different from those of 'normal' showers. The total number of showers is higher than that estimated from a period immediately following the burst on 23 October by $18\% \pm 7\%$, but the lack of gain stability procedures make the estimated number of background events imprecise. Our well separated (pitch = 60 m) array of four telescopes enables the showers to be examined in more detail. For example, those showers triggering two or more of the telescopes may be expected to arise preferentially from primary γ rays. This is because of the earlier development of γ -ray initiated showers in the atmosphere, resulting in a slightly wider light pool on the ground. During the burst on 23 October, the showers had been separated into two groups: those occurring during a 20% phase interval at the peak of the light curve, and the rest. The latter group was similar in all ways to the showers in the interval following the burst, while the former group was richer in double telescope responses, with a χ^2 chance probability of 1% which could indicate a wider light pool for some of the events. An alternative explanation could be that the onphase events possess a harder energy spectrum but an analysis

of the photon densities recorded by the individual phototubes shows no significant difference between the two groups. The number of higher multiplicity responses during the 15 min burst was inadequate for this comparison, and the strength of the possible burst on 31 October was insufficient to permit a similar analysis.

The light curve of the Crab pulsar at much lower γ -ray energies has been reported by the Caravane collaboration. In early observations, it showed a main and an interpulse of a few milliseconds duration, and in later observations the interpulse was seen to decrease in amplitude. The light curves inferred from our events occurring during the bursts on 23 and 31 October are shown in Fig. 2 with the relative phase provided by the ephemeris used. All of them show a single pulse of similar shape and long duration of 6 ms FWHM. It is not clear at what absolute phase, with respect to the radio, optical and X-ray pulses, the emission of UHE γ rays is to be expected, nor whether interpulses should occur at this vastly higher

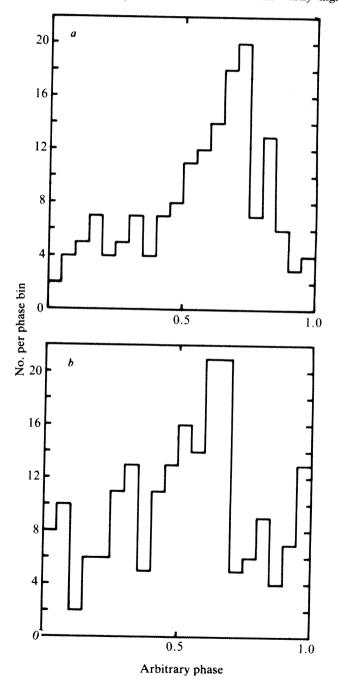


Fig. 2 The light curves for the periods of activity on a, 23 October 1981 and b, 31 October 1981.

photon energy. The derivation of the absolute phase of the light curves in Fig. 2 will require a more detailed ephemeris than that which is now available.

In conclusion, we have shown that the Crab pulsar may be emitting pulsed UHE γ rays in occasional short bursts. A previous balloon experiment¹⁰ at an energy of 1 GeV showed strong variability in pulsed flux between two flights, and during the flight providing the stronger flux, some evidence for variability on a time scale of 1 h. Earlier experiments had reported, at an energy threshold of 2,000 GeV, a time-averaged flux and upper limits near 10⁻¹¹ cm⁻² s⁻¹. The flux of UHE γ rays in the bursts reported above, if averaged over 34 h of observation, suggests a similar time-averaged value and accounts for the previous lack of reproduceability in a straightforward way.

We thank the SERC for funding and for research studentships for I.W.K. and M.W.; Drs T.C. Weekes and H. Tornabene for the loan of equipment; and the commmander and staff of Dugway Proving Ground, Utah for supporting our experimental work.

Received 1 February; accepted 11 March 1982.

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Discontinuous precipitation reaction in the metal of Richardton chondrite

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A metallographic and microprobe study was conducted on the H5 chondritic meteorite Richardton, using material that was free of terrestrial corrosion and ablation heating effects. Our observations indicate that initially well annealed and slowly cooled metal experienced an incomplete episode of discontinuous precipitation in the temperature range 350-400 °C, perhaps after mild deformation at some of the metal-silicate interfaces. There were no indications of externally imposed deformation after the 350 °C episode. The mechanism of discontinuous precipitation rather than plane interface growth has implications for the rate of formation of the metallographic structures, and the apparent temperature of this final heat treatment has implications for proposed reheating events identified by isotopic measurements of the silicate minerals that accompany the metal in this meteorite.

The meteorite was observed to fall in 1918 and was originally catalogued1 in terms of its macroscopic features as a veined spherical olivine-bronzite chondrite. In modern terminology this corresponds to the H family of high iron content. The microstructural condition of its silicate minerals has been reported as appropriate to petrological type 5, which Dodd3 has equated with a metamorphic temperature in the range 700-

800 °C, followed by slow (1 °C My⁻¹) cooling to below 500 °C.

Affiatalab and Wasson⁴ measured the Ni and Co content of α , kamacite and γ taenite, in Richardton. Their α : γ relationship crossed the tie-lines derived by Sears and Axon5 for unreheated H chondrites and was thus more appropriate to the reheated material examined by Sears and Axon5. However, Affiatalab and Wasson4 did not consider the possibility of reheating effects in their sample.

Turner et al.6 investigated the argon release from whole-rock samples of Richardton and derived a total argon release age of 4,500 ± 30 Myr. However, they noted that "significant small fluctuations in apparent age occurred over the extent of the release pattern". This observation might suggest some sort of reheating episode.

By contrast, Evensen et al.7 obtained a Rb/Sr age of 4,390 ± 30 Myr from a study of separate chondrules which they removed from the meteorite.

Thus, from isotope studies of the silicates in Richardton there are indications of reheating additional to that encountered in other H chrondrites, but so far there has been no special metallographic study of possible reheating effects as shown by the metal.

Metal in Richardton is usually present with troilite, FeS, and may be encountered either as very small (10 μm) particles embedded within the silicate chondrules, or, as larger (>100 μm) bodies, approaching chondrule size, in the 'matrix' between silicate chondrules.

Bevan and Axon8 have drawn attention to the existence of metallic chrondrules as a feature of the pristine agglomeration structure of chondrites. Metallic chondrules may contain varying proportions of sulphide and silicate phases and thus they complement the spectrum of silicate/metal proportions and structures that have been encountered in the well-recognized silicate chondrules. However, metal is more responsive than silicate to metamorphic forces, thus the matrix metal may be subject to changes of shape by plastic deformation and to changes of shape and size and α : γ structure by diffusive mass transfer. Hence the chondritic nature of matrix metal may become obscured more rapidly than the silicate structures.

The present study was conducted on metal of the larger (= matrix) type. The samples were from unused residual material from the work of Evensen et al.7 (presented by O'Nions). They were mounted as whole-rock samples for metallographic, electron probe analysis and SEM investigation.

Figure 1 shows a typical area of metal as it appears after etching in Nital. The main features of this structure are a new generation of smaller transformation grains of T-kamacite (low Ni, α, b.c.c. Fe-Ni) growing inward from the metal-silicate

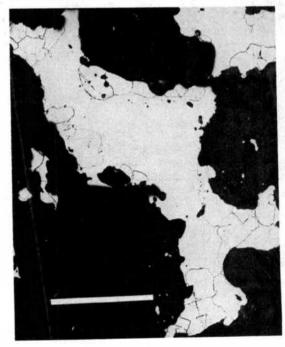


Fig. 1 The structure of a typical matrix metal area in the Richardton (H5) chondrite. Scale bar, 100 µm. At the metalsilicate edge of the large grain of parent kamacite, ~25 smaller grains of transformation structure have developed. There is a small-scale precipitation of γ taenite within the transformation structure; see Fig. 2.

boundary and into the pre-existing monocrystal mass of parent P-kamacite, see Fig. 2. Both P- and T-kamacite are remarkably free from Neumann bands.

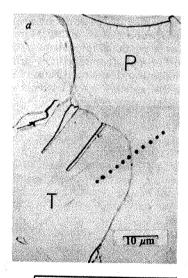
Figure 2 shows that the growth of T has ceased and a parenttransformation interface is detectable. The transformation kamacite is decorated with small elongated particles of taenite (high Ni, y, f.c.c. Fe-Ni) which have developed perpendicular to the parent-transformation (P-T) reaction front. This structure is essentially that of discontinuous precipitation of the type9

P-kamacite → T-kamacite + taenite

The location and distribution of the T grains suggests that they always nucleated at points of local strain on the silicate-metal interface. It appears that cold metal had been subjected to deformation at high-spots in the surrounding silicate, but it is not certain whether the deformation was induced by externally applied forces or was generated by differential contraction between metal and silicate. However, the stress-induced nucleation of the transformation product is confirmed by the occasional appearance of polygonized strain lines within the parent kamacite where it comes into contact with large, unbroken, silicate bodies.

Note that there are additional strain lines that appear to have been formed by the strain effects that accompanied or were generated by the actual progress of the reaction, but this strain is different from that in the nucleating process that we propose above. Some of these considerations have been reviewed elsewhere 10 for the case of manmade alloys.

As usual with chondritic metal¹¹, there is some scatter of bulk nickel content between different individual grains of Pkamacite. However, within each single P+T-kamacite assemblage of Richardton there is a distinct change of nickel content



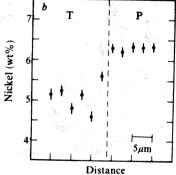


Fig. 2 An enlarged view (a) of the interface between P-(parent) and T-(transformed) kamacite, with the location of microprobe analyses and corresponding (b) Ni contents across the P-T interface. The elongated precipitates perpendicular to the P-T interface are y taenite containing ~50% Ni.

at the transformation interface. The microprobe trace of Ni content across the transformation interface is illustrated in Fig. 2. The small taenite precipitates usually give microprobe analyses in the range 49-50% Ni with a maximum of 52% Ni. The proportions of T-kamacite to precipitated taenite are visually estimated as sufficient to maintain a nickel mass-balance between the parent kamacite and the transformed regions. Reference to the Fe-Ni equilibrium diagram¹² indicates that a taenite of 50% nickel is the equilibrium precipitate at temperatures below ~400 °C.

Thus the composition and microstructure of the matrix metal in Richardton suggests that it developed a shock-free structure at a temperature appropriate to the metamorphic type 5 of its silicates—perhaps 700-800 °C. Slow cooling to the temperature of maximum α solid solubility (~450 °C) allowed the development of P-kamacite with a nickel content at least approaching that maximum.

Below 450 °C cooling may have followed one of two routes, of which we prefer the second. (1) The decreased α solid solubility below 450 °C brought the P-kamacite into a supersaturated condition from which small particles of taenite precipitated in a discontinuous mode as cooling continued. The cellular growth which is visible in Figs 1 and 2 leaves unresolved the mechanism whereby nucleation was induced if the cooling is assumed to have been smooth and continuous. (2) Alternatively, the supersaturated, monocrystal, P-kamacite may have been mildly stressed at locations where relatively large and unbroken bodies of hard silicate made contact with and were able to deform the metal. The temperature of this event cannot be specified but must have been in the low temperature, cold working, range. The deformation was of a slow and gentle nature. The absence of Neumann bands indicated that it was not produced by shock. The evidence lies in the local development of transformation where kamacite is in contact with coarse unbroken silicate bodies but not where it abuts fine matrix material and in the occasional presence of un-recrystallized strain markings around penetrative silicate bodies in the parent

Reheating to 350-400 °C after strain at a lower temperature gives a more unstable condition in the kamacite than is obtained on continuous cooling to that temperature, hence our preference for this production route. In the case of reheating, the taenite precipitates are to be contrasted with the isothermal taenite produced¹³ at higher temperatures in reheated Cañon Diablo kamacite.

The discontinuous precipitation process, now identified for the first time in the cosmic metal of Richardton, involves diffusion within and parallel to the moving transformation front, whereas the previously recognized production of clear or zoned taenite involves diffusion perpendicular to the growth interface. The time required for the production in Richardton of the discontinuous precipitation structure is probably less than would be required for plane interface growth but, nevertheless, appears to be considerable and, if more detailed quantitative information were available an estimate might be attempted. Finally, there have been several studies of meteoritic material artificially reheated at 500 °C and above 13,14 but very little investigation at lower temperatures. To study this possibility we have examined samples of Canon Diablo annealed for 4 weeks at 450 °C and 400 °C. At 400 °C Neumann band alteration was detectable within the mass of the sample but, in addition, at 450 °C there was surface recrystallization, similar to that in Fig. 1 but on a smaller scale, at the cut, and hence deformed edges of the annealed sample.

Received 14 December 1981; accepted 15 March 1982.

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Distribution and enantiomeric composition of amino acids in the Murchison meteorite

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Early determinations of the amino acid distribution in water extracts of the Murchison meteorite revealed unusual amino acids, including isovaline (Ival), α -aminoisobutyric acid (α -Aiba) and pseudoleucine (Ple), as well as common ones such as glycine (Gly), alanine (Ala) and glutamic acid (Glu)¹⁻¹⁰. Amino acids that could be resolved into their respective D- and L-enantiomers were reported to be racemic (see, for example, refs 1, 3, 5), although small sample size might have hindered the precise determination of D/L values. Using improved chromatographic and mass spectrometric procedures we have now been able to amplify and resolve the partially racemized amino acids, Glu, aspartic acid (Asp), proline (Pro), leucine (Leu) and Ala, in an interior sample of a Murchison meteorite stone. Water-extractable amino acids were more racemized than those recovered by digesting the water-extracted meteorite in 6 M HCl. The amino acid composition of this stone was similar to previous reports¹⁻⁸, including the absence of tyrosine (Tyr), methionine (Met), phenylalanine (Phe) and only minor traces of serine (Ser) and threonine (Thr)9. Serine and Thr are usually considered to be terrestrial contaminations. This is the first report of amino acids in a carbonaceous meteorite which, based on currently accepted criteria, appear to be indigenous but for unknown reasons are not racemic. Confirmation of these findings by other investigators suggests that further examination of amino acids in clean carbonaceous meteorites could be beneficial.

We have analysed a single Murchison meteorite stone (Me 2640, Field Museum of Natural History, Chicago, Illinois: 50.7 g) which has over 90% of its surface covered with an intact fusion crust. Standard precautions were taken to prevent laboratory contaminations 11-13. First, the entire surface (~2 g) of the stone was removed with a high-speed drill. Several interior fragments (39.0 g) were ground to a fine powder using a porcelain mortar and pestle. The meteorite powder and a procedure blank were water-refluxed for 8 h at 100 °C. The water extracts were hydrolysed in 6M HCl for 24 h at 100 °C. The procedure used for cation exchange and derivatization of the water-extractable amino acids has been reported previously¹³. To determine whether hydroxy amino acids, such as Ser and Tyr, were present, the N-PFP, O-PFP-(+)-2-butyl esters were converted to N-PFP, O-acetyl-(+)-2-butyl esters^{14,15} for analysis on effective 61-m long 0.5 mm internal diameter nickel capillary columns coated with Carbowax-20M. Next, the water-extracted residue was dried and then digested in 6M HCl for 24 h at 100 °C. In a control experiment¹³, HCl digestion did not racemize or destroy amino acids in the presence of iron oxides and silicate minerals. The Murchison acid extract was desalted using an anion exchange column followed

by a cation exchange column^{15,16}. The amino acids were then derivatized to N-PFP, O-PFP-(+)-2-butyl esters.

Gas chromatograms of the Murchison water and 6M HCl extracts and of the procedure blank are shown in Fig. 1. Amino acid identifications were confirmed by retention times and coinjection of amino acid standards. The hydrolysed water extract was re-analysed by combined gas chromatography-chemical ionization mass spectrometry (GC-CIMS). Amino acid identifications were confirmed by comparing unknown mass spectra with mass spectra of derivatized amino acid standards (see, for example, Fig. 2). Amino acids recovered by digesting the water-extracted meteorite powder in 6M HCl were, as previously reported¹, less abundant than those removed by water extraction, but the samples were too small to confirm their identities by GC-CIMS. Instead they were identified by co-injection and retention times of amino acid standards whose identifications were confirmed by GC-CIMS.

Amino acid D/L values are listed in Table 1. Some amino acids, such as L-Asp and y-amino-n-butyric acid (GABA), co-eluted; a small, unknown peak co-eluted with D-Pro. For D/L values of carbonaceous meteorites to be significant, the purity of individual amino acid enantiomers must be confirmed by a technique that can subtract co-eluting compounds. Selective ion monitoring (ref. 17 for example) made it possible to remove interfering GC peaks and to obtain and confirm D/L values for Asp, Pro, Glu and Ala in the hydrolysed Murchison water extract. For example, three ions characteristic to Asp (m/e = 420, 280, 262) in CIMS were not present in the overlapping GABA mass spectra. The D/L values for selected ion traces were determined from ion current peak heights. Good correlations were found between D/L values of Ala and Glu determined from integrated GC peak areas and from relative ion intensities. As predicted, the D/L value for Asp was slightly higher (0.30) and that for Pro slightly lower (0.30) when peak interferences were eliminated (Table 1). Figure 3 shows traces of intensities of the three ions common to Ala and not present in overlapping or adjacent amino acid GC peaks of the Murchison water extract.

In these experiments, most of the water-extractable amino acids were partially racemized, whereas the 6M HCl-extractable amino acids were less racemized. These results may not contradict previous reports, because we have analysed a large interior Murchison sample (39.0 g), ensuring recovery of an abundant amino acid fraction for enhanced gas chromatograms as well as for the GC-CIMS ion traces.

The abundances of amino acids detected in the hydrolysed Murchison water and 6M HCl extracts are listed in Table 2. We detected none of the common protein amino acids such as Ser, Thr, Tyr, Phe and Met, that are usually absent in carbonaceous meteorites, in these extracts, although minor traces of Ser and Thr were detected by Cronin and Pizzarello (personal communication) in this stone. Serine is the, or one of the, major components of fingerprints, whereas Thr, Tyr, Met, Phe, Glu, Asp, Pro, Leu and Ala are much less abundant. This suggests that originally the stone did not contain Ser, Thr, Tyr, Phe and Met, and that the other amino acids are not fingerprint contaminations. The four most abundant amino acids in the hydrolysed water extract of the Murchison meteorite were α -Aiba, Gly, Glu and Ala. Cronin et al. have reported similar findings

The absence of Ser, Tyr, Phe and Met in the Murchison meteorite extracts might have been due to their decomposition during sample extraction. To investigate this possibility, we prepared duplicate mixtures consisting of ~0.3 mg of Phe, Tyr, Ser, Met, Glu and Ival. Two hundred milligrammes of the unextracted Murchison meteorite powder were added to one of the mixtures; the other mixture served as a control. Water (1 ml) was added to both mixtures, which were extracted and then analysed for their amino acid contents using the procedure described above for the analysis of the Murchison meteorite water extract. The quantities of Phe (52%), Tyr (13%), Ser (45%) and Met (35%) lost from the mixture containing the meteorite powder during extraction and/or cation exchange

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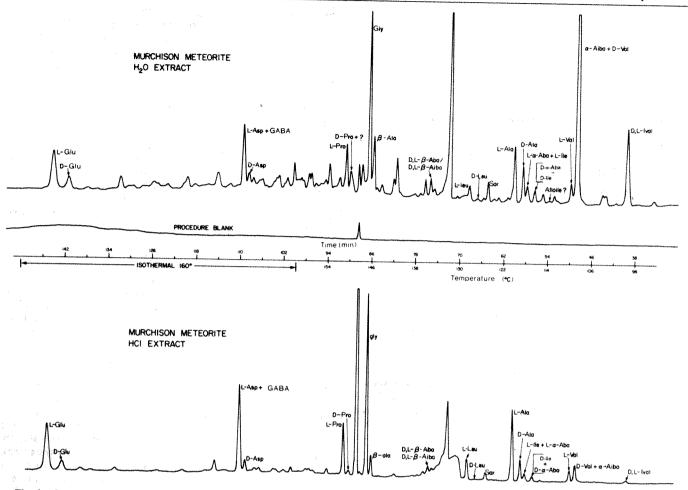


Fig. 1 Gas chromatograms of the procedure blank, water and 6M HCl extracts of the Murchison meteorite. In addition to co-injection of amino acid standards and retention times, all amino acid identifications for the Murchison water extract were confirmed by combined GC-CIMS. The GC and GC-CIMS conditions were identical to those previously reported 13.

were similar to those detected for Glu (40%) and Ival (45%), indicating that if Phe, Tyr, Ser and Met were present in this meteorite stone, they would probably have been detected. Also, there was no deviation from the original D/L values in the control experiments.

The GC and GC-CIMS techniques did not resolve D- and L-Ival. The co-elution of D- and L- α -amino-n-butyric acid (α-Aba) with D- and L-isoleucine (Ile) and their low concentrations made it impractical to use selective ion monitoring to determine their D/L values. Cronin et al. 18 have reported that some amino acid constituents of carbonaceous meteorites, such as Ival and α -Aba, are susceptible to decomposition reactions, which appear to correlate directly with increased residence time of the meteorite on Earth. To resolve the respective D- and L-enantiomers of Ival and α -Aba from the Murchison meteorite, a 3.0 g sample of the Murchison meteorite powder was refluxed twice in water for 2 h at 80 °C. The samples were centrifuged, and the extracts (I and II) were evaporated to dryness and cation exchanged, and then redissolved in water. A slightly modified HPLC procedure 19 was used to isolate and resolve the D- and L-enantiomers of Ival and α -Aba from the two extracts. Isovaline, as previously reported²⁰, was racemic in extracts I (D/L = 0.99 ± 0.06) and II (D/L = 0.99 ± 0.04). α -Aba appeared to be racemic (extract I, D/L = \sim 1.0; extract II, $D/L = 1.13 \pm 0.23$) although its low concentration in the extracts made precise determination of D/L values impossible (Fig. 4).

Some of our results have been independently confirmed in other laboratories. Amino acid abundances in a hot water extract (24 h, 110 °C) of part of the same Murchison meteorite stone (Me 2640) examined by ion-exchange analysis with fluorometric detection (J. R. Cronin and S. Pizzarello, personal communication) were reasonably consistent with those in Table

2. Sample Me 2640 seemed to contain amino acids typical of the Murchison carbonaceous meteorite stones. Fingerprint amino acids, such as Ser, were in low concentrations, and Tyr, Met and Phe were absent. The amino acid abundances and distribution pattern seem virtually to rule out contamination from handling or microorganisms. It is interesting that this stone was enriched in α -substituted amino acids, for example Ival, 2-amino-2,3-dimethylbutyric acid and 2-methylnorvaline, and had fewer amino acids with an α -hydrogen atom than another Murchison stone.

Table 1 Murchison meteorite amino acid D/L values										
Extract	Glu	Asp	Pro	Leu	Ala					
H_2O^*	0.322	0.202	0.342‡	0.166‡	0.682‡					
H ₂ O†	(± 0.044) 0.30	(±0.005) 0.30	(± 0.065) 0.30	(±0.021) ND	(±0.062) 0.60					
HCI*	(±0.02) 0.176 (±0.013)	(±0.04) 0.126 (±0.004)	(±0.02) 0.105 (±0.017)	0.029 (±0.002)	(±0.03) 0.307 (±0.010)					

The experimental errors for the D/L values are shown in parentheses. Approximately 2.0% of the D-amino acids used to obtain the D/L values may be attributed to (-)2-butanol impurities and racemization during 6M HCl hydrolysis. ND, poor resolution did not permit calculation of this value by selected ion traces.

* D/L values were calculated from integrated peak areas and are an average of at least three gas chromatographic determinations.

 † D/L values were calculated from selected ion peak heights and are an average of D/L values determined from three specific ions monitored for each amino acid.

 \ddagger One or more D/L values determined by weighing the peaks (in addition to digital integration).

Table 2 Murchison meteorite amino acid concentrations

Extract	Glu	Asp	GABA	Pro	Gly		nmol g ⁻¹ β-Aiba β-Aba	Leu	Sar	Ala	Ile α-Aba	Val	α-Aiba	Ival
H₂O	18.15	8.48	6.28	13.45	45.79	13.07	5.22	1.91	4.69	15.34	5.98	8.59	107.84	23.57
HCl	6.80	4.02*	3.22*	3.35	14.12	1.25	0.96	0.88	0.57	4.84	0.89	1.09*	1.82*	0.20

 D_L - β -Aba and D_L - β -Aba and D_L -Ile and D_L -a-Aba co-eluted and could not be resolved by GC or selected ion currents because of low abundances and the similarity of mass spectra, respectively. Their respective abundances are reported for the Murchison extracts as average contributions of the two.

A portion of this Murchison meteorite stone and a blank were extracted with hot water and derivatized (N-PFP, O-PFP-(+)-2-butyl esters) by Cronin and Pizzarello (personal communication), and then sent to P. E. Hare for independent GC determinations of the amino acid D/L values. A Perkin-Elmer 900 gas chromatograph with a Perkin-Elmer nitrogen detector, equipped with a 50.0 m×0.3 mm i.d. glass W.C.O.T. chirasilval capillary column, was used. The blank contained only one very small peak with a retention time close to Gly. The D/L values in the derivatized hot water extract of the meteorite were D/L Ala = 0.79, D/L Pro = 0.65 and D/L Glu = 0.56 (P. E. Hare, personal communication).

Only 24 naturally occurring peptides and proteins (3-31 residues long) have been reported not to contain Ser, Tyr, Phe and Met. All are very uncommon in organisms; for example antifreeze glycoprotein 3 (31 residues, isolated from the fish

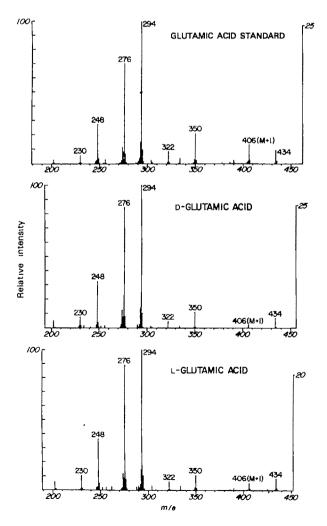


Fig. 2 Mass spectra of glumatic acid standard (top), and of D-glutamic acid (middle) and L-glutamic acid (bottom) in the water extract of the Murchison meteorite. GC-CIMS conditions are given in ref. 13.

Trematomus borchgrevinki), fibrinopeptide B (10 residues, isolated from rhinoceros) and thyroliberin (3 residues, isolated from pig) (M. O. Dayhoff, personal communication). If the Murchison stone we analysed was more than minimally contaminated, it would probably have contained significant quantities of Ser, Tyr, Phe and Met.

The presence of L-Ser in carbonaceous meteorites is generally considered to represent a terrestrial contribution. Assuming that small quantities of L-Glu, L-Ala and L-Pro were introduced into the Murchison stone at the same low level as Ser (0.9 nmol g⁻¹; Cronin and Pizzarello, personal communication), the D/L values can be recalculated after decreasing the L-amino acid abundances proportional to the trace amount of Ser found in this sample. This correction, however, does not significantly alter the D/L values.

Several hypotheses may at least partially account for the occurrence of non-racemic amino acids in this Murichson meteorite stone: (1) terrestrial contamination during entry, impact, collection and/or in the laboratory; (2) various unknown preferential syntheses of non-racemic amino acids during laboratory procedures; and (3) unknown stereoselective chemical synthesis and/or decomposition reactions unrelated to terrestrial sources.

(1) If the stone was more than minimally contaminated before or during impact and/or at any time thereafter, the absence of Tyr, Phe, Met and only a trace of Ser, would not be predicted, as these amino acids are ubiquitous to terrestrial biogenic systems. Also, the $\delta^{15}N$ values of amino acids and other nitrogenous compounds in Murchison are very different from terrestrial δ^{15} N values²¹. If terrestrial contamination by Lamino acids is invoked to account for the Murchison meteorite D/L values, there is either a very unusual contamination source of specific amino acids, or preferential adsorbtion and/or decomposition of specific amino acids, by interactions at probably ambient temperatures of the meteorite organic/inorganic matrices with the intruding material(s). Although preferential adsorbtion of L-amino acids by mineral matrices has been reported (ref. 22 for example), the adsorption or destruction of L-amino acids such as Ala, Glu, Asp, Pro and Leu in preference to others, for example Ser, Tyr, Phe and Met has not. Amino acids extracted from the meteorite with 6M HCl were less racemized than the water-extractable amino acids. To account for these ratios by contamination, the terrestrial contaminants would have to be preferentially and very tightly complexed to the meteorite matrix. In previous experiments in which rocks were soaked in aqueous amino acid solutions for 1,321 h, all the amino acids entering the samples during soaking were extracted from the rocks using the water extraction procedure we used for the Murchison meteorite. Subsequent acid extraction resulted in no additional recovery of amino acids inserted into the rock samples (M. H. Engel and B. Nagy, in preparation). If the Murchison meteorite was contaminated with L-amino acids they should have been removed during the water extraction. L-amino acids introduced into the stone on Earth during the 11 yr before analysis would be predicted to be less tightly bound to, and more readily extracted with, water from the meteorite organic/inorganic matrix than amino acids incorporated in the meteorite some 4.5×10° yr ago. Yet, 6M HCI extraction recovered amino acids showing even less racemization than the water extract, so, in fact, the less racemic

^{*} Concentrations were estimated from the relative abundances determined for the Murchison water extract by selected ion monitoring.

amino acids are more tightly held in this stone than those extractable with water. The finding of non-racemic amino acids. however, makes contamination of the Murchison meteorite a

serious possibility.

(2) Hydrogen cyanide polymers²³ and N-carbamyl amino acids7,8 have been proposed as amino acid precursors in the water extracts of carbonaceous meteorites. Hydrolysis of these precursors to free amino acids would not involve reactions at the asymmetric centres of these compounds, indicating, however, that such precursors, if they were present in this stone, were themselves non-racemic.

(3) Mechanisms have been proposed for the stereoselective decomposition of amino acids²⁴⁻²⁷. If the amino acids in the Murchison meteorite were initially racemic, and if these mechanisms prove applicable, they might indeed account for the D/L values observed. A few mechanisms (for example ref. 28) have been proposed for the extraterrestrial stereoselective synthesis of organic compounds, but it is highly unlikely that amino acids in the Murchison meteorite would be completely racemic had they initially been present in the L or D form²

We therefore conclude that the partially racemic amino acids in this Murchison meteorite stone are probably due to extraterrestrial stereoselective synthesis or decomposition reactions, although the possibility of unusual terrestrial contamination cannot be excluded. Different stones of the Murchison meteorite may be heterogeneous with respect to their carbon

compound compositions.

Optical activity in carbonaceous meteorites infer the possibility of asymmetric chemical syntheses unrelated to terrestrial sources. Although we do not know the specific cause(s) of the optical activity, the independent confirmations of our results strengthen our conclusion that the Murchison meteorite sample we studied was clean and that some of the amino acids are partially racemic.

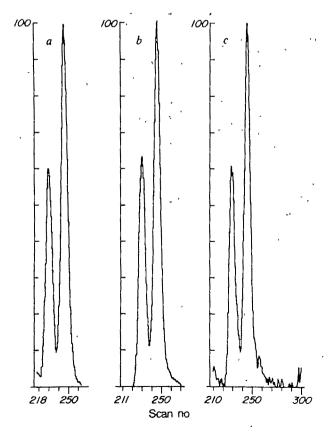


Fig. 3 Selected ion traces for alanine in the water extract of the Murchison meteorite. a, m/e = 218, D/L Ala = 0.60; b, m/e = 216, D/L Ala = 0.63; c, m/e = 320, D/L Ala = 0.58. m/e = 320 is the characteristic M+29 adduct formed by the addition of C₂H₅⁺ to the alanine derivative during chemical ionization.

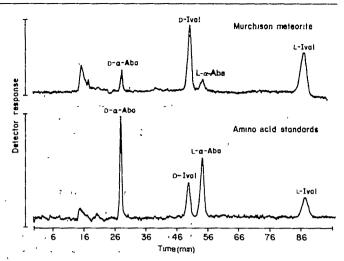


Fig. 4 Chromatograms of the D- and L-enantiomers of isovaline and α -amino-n-butvric acid from extract II of the Murchison meteorite (top) and of isovaline and α -amino-n-butyric acid standards (bottom). D, L-isovaline and α -amino-n-butyric acid were initially isolated from extract II by HPLC using a 25 cm× 2.0 mm i.d. stainless-steel cation exchange column packed with a 5 um cation exchange resin that had been converted to the pyridine form. An aqueous buffer consisting of pyridine (0.2 M) and formic acid (pH 3.2) was used. The buffer flow rate was 0.12 ml min The column temperature was 53 °C. The amino acids were collected using a stream splitter before reaction with O-phthaldialdehyde and subsequent fluorometric detection. The D- and Lenantiomers of isovaline and α -amino-n-butyric acid were resolved using the procedure developed by Engel and Hare¹⁹ for the resolution of basic amino acid enantiomers.

We thank H. Ogino for helping to determine the relative stabilities of amino acids during sample extraction and to prepare additional Murchison meteorite extracts for analysis by HPLC, S. Y. Chang for help with the GC-CIMS analyses, J. R. Cronin, S. Pizzarello and P. E. Hare for their independent examinations of the meteorite sample, K. A. Kvenvolden, L. Margulis and S. L. Miller for comments and E. Olsen for providing the meteorite sample. This research was supported by NASA grant NGR 03-002-171.

Received 16 November 1981, accepted 26 February 1982

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Structural features of todorokite intergrowths in manganese nodules

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The disordered, cryptocrystalline phases that comprise manganese nodules^{1,2} typically yield broad, diffuse X-ray lines that have led to ambiguity in their identification. Of particular interest is the manganese oxide phase that has been correlated to the presence of copper and nickel in nodules. This host phase has a characteristic X-ray d-spacing of ~10 Å and has been compared with both a naturally occurring material—todorokite^{3,4}—and a synthetic phase—buserite^{5,6}. The structures of those phases have been difficult to decipher, but recent work using high-resolution transmission electron microscopy (HRTEM) has provided insight into the todorokite7 and buserite⁸ structures. Using HRTEM results we now show that the nodule 10 Å phase does have the same structure as nonmarine todorokite9. In addition, structural imaging shows that both nodule and non-marine todorokite exhibit similar disordering phenomena. A unique feature of the nodule todorokite is its intimate and topotactic intergrowth with a possible doublechain oxide. Its paragenetic relationship to todorokite is not yet determined.

By structural imaging of non-marine samples⁷, todorokite was shown to be one of three related intergrowth families that occur in manganese oxides. Each family consists of an intergrowth of variously sized tunnels that differ in sharing either single, double or triple chains of edge-shared [MnO₆] octahedra. Todorokite structures share triple chains, and the large resultant tunnel structures allow for the inclusion of water and large cations. The proposed atomic arrangement of a common todorokite structure is shown in Fig. 1. This tunnel structure

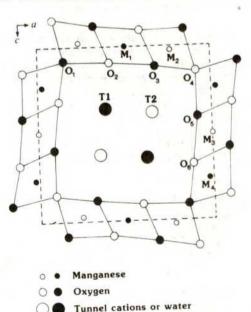


Fig. 1 The proposed atomic arrangement of a common todorokite structure, with three chains of manganese octahedra per side. The structure is projected down the tunnel direction [010]. \bigcirc , b = 0; \bullet , b = 1/2. Dashed line denotes the unit cell.

leads to a fibrous habit, and these fibres are commonly twinned at 120° angles.

Presumed todorokite from manganese nodules has been studied previously by scanning and transmission electron microscopy. Both Siegel¹⁰ and Chukhrov et al.¹¹⁻¹³ found fibrous material in nodules from the Pacific Ocean that contained twinning at the characteristic 120° angle; todorokites with different predominant tunnel sizes were found in these studies. In addition, Chukhrov et al.¹¹ show a high-resolution image of their nodule todorokite viewed perpendicular to the tunnel direction in which different tunnel sizes as well as disordering phenomena are evident.

The nodules we examined were collected from the North Equatorial Pacific near Manganese Nodule Project (MANOP) site S, which is between the Clarion and Clipperton Fracture Zones at about 140° W and 11° N. Details of the morphological and chemical features found in the nodules are presented elsewhere 14. The nodule cores were found to consist largely of moulds of biogenic material such as discoasters, coccoliths and radiolaria. Scanning electron microscope studies of the nodules show that fibres of crystalline manganese oxide occur almost exclusively with the biogenic debris, an association that may have important paragenetic significance. Figure 2 shows

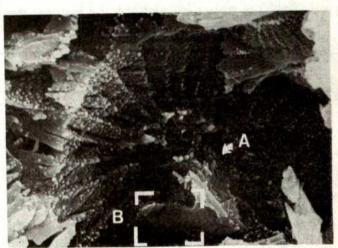


Fig. 2 Close-up of an impression of a coccolith shell. In the centre foreground, fibre tips of todorokite are evident (A). Todorokite fibres overlapping at about 120° angles can be seen in the box B.

examples of overlapping fibres and fibre tips associated with a mould of a coccolith shell. This fibrous morphology is similar to that of non-marine todorokite.

Samples of this core material were crushed and examined in a slightly modified JEOL JEM 100B microscope 15. Due to their fibrous morphology, almost all grains settled onto the microscope grids with their lengths perpendicular to the electron beam direction. A structure image taken in this direction in Fig. 3a reveals triple, quadruple and quintuple chain widths. In other images, double chains, as well as sextuple and septuple chains, are shown. A view down the tunnel direction is necessary to determine the common shared width of these differentlysized tunnels. Unfortunately, the small cross-section of the nodule fibres precludes the use of electron diffraction for this orientation process. In one case, a fibre was fortuitously oriented nearly parallel to the electron beam. Figure 3b shows the image obtained, in which a slightly skewed framework of differently sized tunnels is evident. The estimated tunnel dimensions are labelled in the sketch of Fig. 3c. The image in Fig. 3b confirms that this nodule, 10 Å phase, is formed of an intergrowth of tunnel structures that have a triple chain of manganese octahedra in common. This fibrous 10 Å phase does then indeed have the same structure as non-marine

Disordering phenomena are evident at all levels of

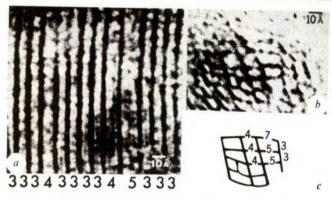


Fig. 3 a, HRTEM micrograph of todorokite from a nodule core, viewed perpendicular to the tunnel direction. The tunnels run from top to bottom in this image. Triple chains of manganese octahedra predominate, but quadruple and quintuple chains are also present. Note the bending of the tunnels on the right side. b, A nodule todorokite fibre oriented with its length parallel to the electron beam direction. c, Sketch of the centre of b with labelled tunnel sizes. The tunnels all have a triple chain of manganese octahedra in common.

magnification of the nodular todorokite material, and they contribute to the streaking of the todorokite diffraction patterns. In some samples, the tunnel structure is disrupted by faults along the chain length. Figure 4a, shows a quadruple chain tunnel becoming a triple chain at a fault. The adjacent triple chains are displaced below the fault. Similar disruptions are evident in the HRTEM image of Chukhrov et al.11 On a larger scale, narrow fibres are gently warped. A distinct bend in a fibre is seen in Fig. 4b. Similar phenomena are found in non-marine todorokite.

In some sample grains, a different phase was seen topotactically intergrown with the todorokite. This other phase gives fringes between 4.5 and 5 Å in width. This spacing may be obscured in X-ray diffraction patterns due to its similarity to a common spacing in todorokite. The identity of this nodule 5 Å phase is uncertain, but structural images obtained from it resemble those we have previously acquired from the doublechain manganese oxides-ramsdellite and groutite. In some cases, todorokite and this 5 Å phase are evident in the same image. In other samples, only the 5 Å phase is seen, and it is associated with apparent relict fibres of todorokite (Fig. 5a). In Fig. 5a, the large, 100 Å-wide fibres resemble todorokite in the way they are twinned at 120° angles. However, high-resolution imaging shows only amorphous regions and fringes from the 5 Å phase (Fig. 5b). It is possible that fibres of todorokite altered to the 5 Å phase. The chemistry of the 5 Å material is

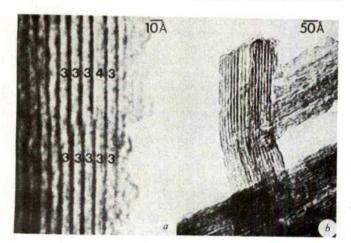


Fig. 4 Disordering phenomena in todorokite. a, A disruption along the tunnel length. A quadruple chain tunnel merges to a triple chain. b, A bent fibre of todorokite.

uncertain at present, but preliminary work has indicated that iron is not present. The chemistry will be important in determining the genetic relationship between the todorokite intergrowths and the 5 Å phase.

This study indicates that further use of HRTEM in the study of the cryptocrystalline manganese nodule phases is warranted both to confirm earlier deductions from X-ray work and to find new phases and their detailed structural features. Such structural information should eventually provide clues to longstanding problems regarding the origin and accretion of nodules. For instance, different environments of formation of nodule todorokite may influence their predominant tunnel size. In the nodule todorokite samples of Chukhrov et al. 11-13, a predominant [100] width is 14.6 Å which corresponds to quintuple chains of manganese octahedra, whereas in our samples the predominant width is of triple chains. Perhaps a layer 10 Å phase (analogous to buserite) occurs in other environments. Two other manganese oxide phases—birnessite and vernadite have been proposed to occur in nodules. Both these structures are poorly understood and HRTEM should provide insight into their nature. A final problem is the location of transition elements such as Cu, Ni and Co in nodule phases. Use of structural imaging with concurrent microanalysis should firmly establish the identity of the host phases.

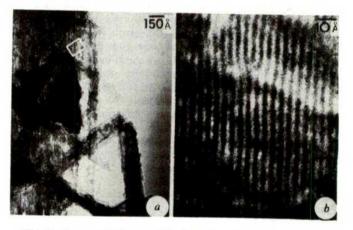


Fig. 5 Images of the possible double-chain phase. a, Fibres twinned at 120° are evident. b, Close up of box in a shows fringes of the possible double-chain phase.

We thank S. Selkirk for aid in drafting Figs 1 and 3c, Dr J. E. Post for reviews, and K. Jones and E. Seeling for assistance with the scanning electron microscopy. M.S. thanks Drs H. D. Holland and R. Burns for guidance and support. This research was supported by grants EAR79-26375 and EAR 79-04364 from the Earth Sciences Division of the NSF and the NSF-1D0E Seabed Assessment Program Manganese Nodule Program (grant no. 832550CE). The high-resolution imaging work was performed in the HREM Regional Facility in the Center for Solid State Science at Arizona State University.

Received 14 December 1981; accepted 8 March 1982.

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Pulsating flow of a plastic fluid

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It has been observed^{1,2} that when a polymeric solution flows through a circular pipe under a pressure gradient which varies periodically about a non-zero mean, the mean volumetric flow rate increases over that which is due to the mean pressure gradient alone. The magnitude of this flow enhancement depends on the fluid properties and is a function of the mean pressure gradient and the amplitude and the frequency of its varying component. Theoretical work¹⁻⁶ has indicated that this phenomenon is due to the shear-thinning property of the polymeric fluid. More generally, the flow enhancement arises because of the non-linear relationship between the stress and the strain rate which causes the effective viscosity to decrease. Indeed, we show here that a flow enhancement is possible for a Bingham fluid⁷.

This fluid does not deform below a certain yield shear stress $\tau_{\rm v}$; above this yield shear stress it flows like a newtonian fluid (with constant viscosity) and is thus not shear-thinning in the normal sense. However, a power analysis shows that the extra power required to pulsate the flow is greater than that required to generate the extra flow enhancement under a steady pressure drop. Consequently there is no economic reason to pulsate the flow. The set-up of the experimental rig is shown in Fig. 1. A peristaltic pump B was used to circulate the test fluid from a reservoir A which was immersed in a thermal bath. The temperature was regulated to ±1 °C. Pressure gradient noises in the pump were effectively damped out using two smoothing bottles C in series. The pulsatile pressure gradient was generated by a piston E driven by an offset circular cam D. High frequency components of this pulsatile pressure gradient were damped out using another smoothing bottle F. In this arrangement the fluid flowed under a pressure gradient of the form

$$\partial P/\partial z = -P_0(1 + \varepsilon n(t))$$
 (1)

where P_0 is the mean pressure drop per unit length, n(t) is a near perfect sinusoidal function of time and ε is the relative amplitude of the pulsatile pressure gradient. To measure P_0 , a

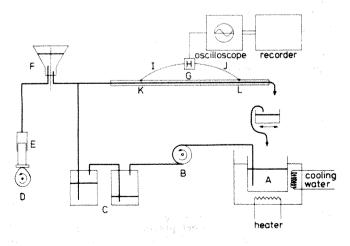


Fig. 1 Schematic arrangement of the pulsating flow experiment.

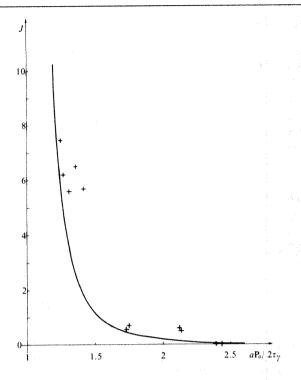


Fig. 2 The base flow enhancement. Solid line is the theoretical curve based on equation (5). +, Data on a 10% bentonite solution taken at a frequency of 0.1 Hz, temperature (24.5 ± 0.5) °C and the amplitudes (ϵ) vary between 0.3 and 0.4.

differential pressure transducer H connected to the test section G at K and L was used. To avoid the attentuation in the amplitude of the pulsatile pressure gradient, we measured ε by mounting the transducer H directly on K and disconnecting the plastic tube J. The efflux was measured by the conventional beaker and stopwatch method.

The test fluid is an aqueous suspension of bentonite at 10% by weight. The bentonite powder (manufactured by Magcobar) conformed to API specification 13A. On a wet screen analysis it leaves a maximum of 4% residue on a US standard sieve no. 200 (nominal opening of $74 \mu m$). At low shear rate the bentonite suspension behaves essentially like a Bingham fluid. That is, in a simple shear flow of shear rate $\dot{\gamma}$, the shear stress is given by (note that τ is an odd function of $\dot{\gamma}$)

$$\tau = \eta \dot{\gamma} + \tau_{v} \operatorname{sign} (\dot{\gamma}) \tag{2}$$

where sign $(\dot{\gamma}) = 1$ if $\dot{\gamma} > 0$ and sign $(\dot{\gamma}) = -1$ if $\dot{\gamma} < 0$. Using a commercial vane viscometer (Wykeham Farrance Engineering Ltd) and an Instron Model 3250 Rotary Rheometer with a cone (2°) and plate (2 cm radius), we found that $\tau_y = (6.4 \pm 0.5)$ Pa.

To quantify the effect of the pulsatile pressure gradient, we define a flow enhancement I as

$$I = (\langle Q \rangle - Q_0)/Q_0 \tag{3}$$

where $\langle Q \rangle$ is the average flow rate (the angular brackets denote a time average) and Q_0 is the flow rate due to the mean pressure gradient alone.

At low value of ε (ε < 0.4) and keeping the mean pressure drop and the frequency of the pulsatile pressure gradient fixed, we found that I is proportional to ε^2 , which was already noted by Barnes et al.^{1.2} for polymeric liquids. However, for the bentonite solution tested we found I to be independent of the frequency ω of the pulsatile pressure gradient, up to a value of $\omega \sim 1$ Hz. The experimental findings at $\omega = 0.1$ Hz are dis-

played in Fig. 2 where we plotted the base flow enhancement $J = I/2\varepsilon^2 \langle n^2 \rangle$ against a dimensionless pressure drop $aP_0/2\tau_{\gamma\gamma}$ where a = 3.2 mm is the inner radius of the test section. (Note that $2\langle n^2 \rangle = 1$ for a sinusoidal n(t).)

To compare the experimental findings with the theory based on the Bingham fluid model we use equations (1) and (2) in the equations of motion neglecting body forces and the fluid inertia. The latter assumption is reasonable in view of our Reynolds number (based on ω and a) is always <0.01. It can be easily shown that the flow enhancement is given by

$$I = 2\varepsilon^2 \langle n^2 \rangle J + o(\varepsilon^2) \tag{4}$$

where

$$J = (\phi^4 - \frac{4}{3}\phi^3 + \frac{1}{3}), \qquad \phi = aP_0/2\tau_v \tag{5}$$

Equations (4) and (5) show that the theoretical flow enhancement is independent of the frequency and that it is proportional to ε^2 for small ε^2 . Furthermore a knowledge of the yield shear stress (and not the viscosity η) and the mean pressure drop per unit length is sufficient to determine the base flow enhancement J. The theoretical base flow enhancement is plotted in Fig. 2. Clearly, in view of a large experimental error which must exist when taking the difference between two quantities of the same order of magnitude, $\langle Q \rangle$ and Q_0 , the agreement between theory and experiment is very satisfactory.

To find out whether pulsating a pipe flow of a Bingham fluid is also economically viable we must also look at the net power requirement. Now the net power required to pulsate the flow is

$$\begin{split} W &= -\left\langle Q \frac{\partial P}{\partial z} \right\rangle \\ &= \frac{\pi a^4 P_0^2}{8 \, \eta} \left(-\frac{4}{3} \phi^{-1} + \frac{1}{3} \phi^{-4} + \varepsilon^2 (1 + \phi^{-4}) \langle n^2 \rangle + \mathrm{o}(\varepsilon^2) \right) \end{split}$$

In steady conditions we need a pressure drop per unit length of

$$P' = P_0(1 + 2\varepsilon^2 \langle n^2 \rangle (\phi^{-4} - 1) + o(\varepsilon^2))$$

to generate a flow rate $\langle Q \rangle$. Thus the power required in steadystate conditions which yields an identical (to $o(\varepsilon^2)$) flow rate is W' = P'(Q). The extra power required to pulsate the flow is

$$\Delta W = W - W'$$

$$= \varepsilon^2 \langle n^2 \rangle (\phi^8 - 4\phi^4 + \frac{8}{3}\phi^3 + \frac{1}{3})(\phi^4 - 1)^{-1}$$

$$\times (\phi^4 - \frac{4}{3}\phi^3 + \frac{1}{3})^{-1} + o(\varepsilon^2)$$

If terms of $o(\varepsilon^4)$ and higher are neglected, ΔW is seen to be always positive. Consequently there is no economic advantage in pulsating the flow of a Bingham fluid. This conclusion does not agree with that of Barnes et al.2 who modelled their polymeric fluids by an Oldroyd model.

This research is supported by an ARGC grant (F 79 1559 7R). We thank the Oil Drilling and Exploration Company for donating the bentonite sample.

Received 12 January, accepted 11 March 1982

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Steam explosions of molten iron oxide drops: easier initiation at small pressurizations

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If a light water nuclear reactor core were to overheat, hot molten materials might contact liquid water and cause a steam explosion damaging to the containment structure¹. To predict such an occurrence and the ensuing damage, we in the Sandia National Laboratories have been performing large² and small^{3,4} scale experiments coupled with analytical modelling^{5,6}. As part of this effort, we have been releasing single drops of a core melt simulant, molten iron oxide, into liquid water. Small steam explosions are triggered shortly afterwards by applying a pressure pulse to the water. The threshold peak pulse level above which an explosion always occurs was studied at ambient pressures between 0.083 and 1.12 MPa. Unexpectedly, the threshold decreased to a minimum in the range 0.2-0.8 MPa and then increased again. The observation that gentle pre-pressurization meant easier triggering should improve the predictive capability of our models and suggest conditions for reducing the hazards of these explosions.

A steam explosion can occur when a hot, molten material contacts liquid water. The explosion, which sometimes generates destructive shock waves, is caused by the fragmentation of the melt and rapid transfer of heat to the water with very rapid generation of steam. Explosions such as these pose serious hazards whenever large quantities of molten materials are handled, for example, in the metallurgical and papermaking industries. (Cronenberg and Benz¹ recently reviewed steam explosions with particular emphasis on nuclear reactor safety.)

Melts that would result from a nuclear reactor core meltdown, called coriums⁷, would be composed of various mixtures of UO2 fuel, zircaloy cladding, and stainless steel structures, oxidized to some extent by contact with steam at temperatures near 2,000 K. We have been studying iron oxide because it can be a major component of some of the coriums⁸; it also simulates some of their physical and chemical properties. Single drops are used because it enables excellent control of the many parameters involved in the interactions.

Individual 2.9-mm diameter pendant drops of iron oxide were heated to 2,230 K with 100-200 W of focused continuous wave CO₂ laser radiation^{4,9}. The drops, supported on the bottom end of a vertical iridium wire, were positioned a few millimetres above the surface of liquid water held in a polymethylmethacrylate tank 152 mm square and 300 mm deep. The tank contained a water heater, stirrer and thermometer. At various distances from the bottom of the tank, a pressure pulse was generated in the water by the capacitor discharge explosion of a submerged wire. The magnitude of the pulse which triggered the steam explosion was varied by raising or lowering the exploding wire. The interactions were analysed using high speed cameras, a lithium niobate pressure transducer 10,11 hung in the water, and by examination of the iron oxide residues after the interactions.

The entire apparatus was enclosed in a steel pressure chamber which had a zinc selenide window12, a transparent fused silica window, and a thick glass window for passage of the laser radiation, pyrometer viewing and photography, respectively. The oxygen partial pressure within the chamber was held at ≈0.02 MPa to maintain the composition of the drop at FeO_{~1.2} (ref. 13). The total chamber pressure was varied from local atmospheric (0.083 MPa) to 1.12 MPa by pressurizing with Ar or N₂. After shaking the molten drop from the wire, the interaction was initiated by exploding the wire \approx 125 ms after the drop entered the water.

The steam explosion produces a steam bubble which alternately grows and collapses for several complete cycles. Each bubble collapse produces a pressure pulse in the water. These pulses, along with the triggering pulse, cause progressively finer fragmentation of the melt, presumably due to collapse of the film boiling layer that surrounds each melt particle at the beginning of each cycle. Bubble growth is caused by the rapid transfer of melt enthalpy to the water with each breakup of the melt. The final debris consists of finely divided iron oxide with average particle diameters in the tens of micrometres range. Further description of the experiments is given in refs 4 and 14.

Recently, we have studied the effects of varying the basic parameters involved in the interactions¹⁴, including ambient pressure. The effects of increased ambient pressure on vapour explosions have been considered previously, both analytically^{6,15–18} and experimentally (several experimental reports are cited in ref. 6). It seems established that for given triggering conditions, it should become progressively more difficult and eventually impossible to initiate an explosion as ambient pressure is increased. However, it has been neither predicted nor observed that initially it becomes easier before it becomes harder to trigger an explosion as this pressure increases, at least for single drops of a melt such as ours.

The easier triggering was first observed as a sharp drop in the threshold triggering peak pressure, Δp , required to initiate an explosion as ambient pressure, P_{∞} , was increased. This is shown in Fig. 1, which indicates the occurrence or absence of an explosion as these two parameters were varied; water temperature was held constant at 297 K. Note the abrupt dip in threshold trigger pressure from $\Delta p \approx 0.4$ to <0.2 MPa as P_{∞} is increased from 0.083 to 0.2 MPa, and the broad minimum in Δp which extends to $P_{\infty} \approx 0.8$ MPa.

As chamber pressure is increased at constant water temperature, the water subcooling also increases. (The subcooling of a liquid is the difference between its boiling temperature at a given ambient pressure and its actual temperature. Subcooling is normally considered to be one of the parameters which governs heat transfer in film boiling situations of the sort that prevail in our experiments.) Because at a given ambient pressure

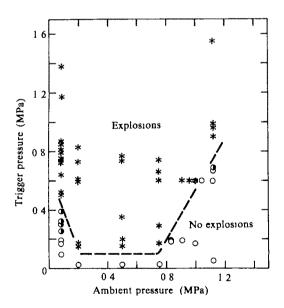


Fig. 1 Plot of peak triggering pulse pressure against ambient chamber pressure for the interaction of a single 2.9 mm drop of molten iron oxide at 2,230 K released into water. Temperature of the water was 298 K for the points at an ambient pressure of 0.083 MPa; for all other experiments, it was 296 ± 1 K. *, Vigorous explosion with fine fragmentation. ①, Sluggish interaction, often with an explosion ≈ 0.1 s after the triggering pulse. O, No explosion.

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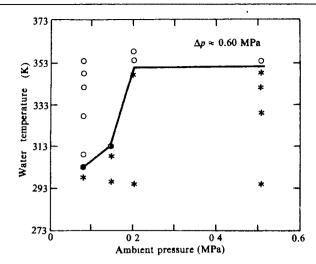


Fig. 2 Effect of water temperature on the interaction between single drops of molten iron oxide and liquid water as ambient pressure, P_{∞} , varies. A constant peak trigger pressure, Δp , of ~ 0.60 MPa is used in all experiments. *, Vigorous explosion with fine fragmentation. \bigcirc , No explosion. *, Duplicate experiments: one drop exploded, the other did not.

steam explosions normally become easier to initiate as water subcooling increases⁶, the question arises as to whether the increased ease of initiation as ambient pressure increases is due primarily to water subcooling.

We therefore performed a series of experiments whereby ambient pressures were increased but water temperatures were also increased appropriately to maintain constant water subcooling. We again found that at an ambient pressure of 0.2 MPa the trigger level required dipped to <0.3 MPa; however, it increased more rapidly than in Fig. 1 to \approx 0.7 MPa at $P_{\infty} = 0.5$ MPa. The increased ease of triggering a steam explosion at increased ambient pressures, therefore, seems to be due only partially to water subcooling effects.

We then investigated the easier triggering as pressure increased by varying water temperature and ambient pressure in a different way, keeping the triggering level fixed at $\Delta p \approx 0.6$ MPa. Because it becomes more difficult to initiate steam explosions as the water becomes hotter at constant ambient pressure⁶, the threshold temperature above which explosions no longer can be initiated for a fixed triggering level becomes another measure of the ease of initiating a given melt-water interaction.

The measured threshold temperatures shown in Fig. 2 were 300 and 310 K for $P_{\infty} \approx 0.083$ and 0.14 MPa, but rose sharply to 350 K for $P_{\infty} \approx 0.20$ and 0.50 MPa. This sharp rise again indicates that triggering the steam explosion of a single drop of molten iron oxide becomes easier at ambient pressures a few times greater than atmospheric.

Although the effect is as yet unexplained we feel that the easier initiation as ambient pressure increases may have an important role in the triggering and propagation of a large scale steam explosion through a coarsely premixed dispersion of melt in water. For example, if a quasistatic localized pressurization should occur in the early stages of the explosion (water temperature would probably remain essentially constant), this would increase the sensitivity of the pressurized portion of the array to subsequent pressure disturbances generated in the system. This may aid the transmittance of the explosive interaction throughout the system.

This work was supported by the US Nuclear Regulatory Commission.

Received 27 October 1981, accepted 19 February 1982

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Optical spectroscopy in a shocked liquid

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Scientists in the USSR have reported chemical effects of shock waves in condensed materials which they cannot attribute to the effects of pressure and temperature. They postulate the existence of "catastrophic" chemical effects in the region of near-discontinuous change called the "shock front"1.2. Direct testing of this postulate requires time-resolved observations of chemical processes as the sample is traversed by the shock, and to this end we report here the development of methods for the measurement of temporal changes in the electronic spectra of condensed materials in such conditions. The time resolution is about 3×10^{-8} s.

Measurements in liquid CS₂ show unexpected increases in reflectivity and absorption as well as large shifts of absorption band edges towards the red at shock pressures of <12 kbar. The significance of these observations is not yet understood, but it is clear that electronic, and therefore chemical, changes are taking place in transient conditions not normally accessible to chemical experimentation.

In 1920, Parsons reported unsuccessful attempts to produce artificial diamonds by compression of carbonaceous materials with shock waves³. Forty-one years later the feasibility of the conversion was established4. Work before 1961 had shown that profound changes could be produced in metals by strong shock waves; later experiments, carried out principally in the USSR, have demonstrated the feasibility of driving a variety of chemical reactions by compression with moderate to strong shock waves^{1,2}. While these experiments, called 'recovery experiments' because analysis takes place only on samples collected after being shocked, were in progress, various types of dynamic shock experiments were developed and used for studying mechanical, thermal, electrical and optical properties of condensed matter⁵. These latter experiments are most successful if plane one-dimensional shock waves are used to compress the samples. Such shock waves can be produced in the laboratory by planar impact of a projectile on the sample.

The oldest known chemical reaction initiated by shock waves is detonation in high explosives. Several years ago, moved by the difficulty of making dynamic measurements in detonation, Sheffield undertook dynamic measurements on a non-explosive material, liquid CS₂, to establish its decomposition, to provide evidence that dynamic measurements in such a system are possible and to measure kinetic parameters of the process^{6,7}. The success of his measurements led to a desire for measurements more directly related to chemical processes and thus to the development of spectroscopic techniques reported here.

Optical spectroscopy in shock experiments has been attempted before but with qualified success⁸⁻¹¹. In our experiments, a liquid sample is contained in a penny-shaped cell, bounded front and back by transparent sapphire disks and on the sides by a cylindrical brass shell. The shock is created by impact of a flat-faced projectile on the plane face of one of the sapphire disks. Just before impact a xenon flash lamp is pulsed, producing a spectrally broad light which traverses the sample assembly, passes through a grating spectrograph and is recorded on 35 mm film by means of a rotating mirror streak camera. The record on the film has wavelength varying across the film strip, time varying along the strip and density varying with reflectivity or transmissivity of the sample. For reflection experiments the light source is external to the target and projectile assemblies. Light is brought into the cell through the sapphire disk opposite the impact side and in a direction very close to normal. It comes out near the normal and is reflected through the entrance slit of the spectrograph. For transmission experiments the flash lamp is mounted in the projectile. Contacts on the face of the projectile connect with high voltage leads in the target just before impact. The power source is triggered soon afterwards and light transmitted through the cell is reflected

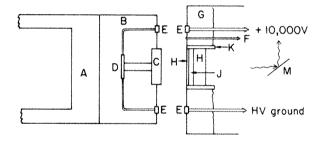


Fig. 1 Schematic representation of light transmission experiment. A, aluminium body of projectile; B, PMMA/epoxy projectile head; C, sapphire impactor; D, Xe flash lamp; E, high-voltage connectors; F, trigger pin for oscilloscopes; G, epoxy target body; H, sapphire plates of test cell; J, cavity containing liquid CS₂; K, brass container for test cell; M, mirror which reflects light into objective mirror of spectrograph.

into the spectrograph as before. The subject material has been CS₂ at room temperature and an initial pressure of 1 atmosphere. Thicknesses of the CS_2 layer vary from $\sim 150 \,\mu m$ to < 1 µm, and final pressure in the sample is reached by reverberation between the sapphire plates. Reflection records obtained thus far are in the UV, approximately centred on the transmission region at 2,700 Å between two absorption bands¹². The records show transmission as expected before the shock reaches the sample and momentary extinction as the shock enters the sample, followed by reflection all across the film as pressure in the sample rings up to its final value. Reflection coefficients are estimated to be >0.2 at 80 kbar and >0.6 at 120 kbar. At a final pressure of 80 kbar, reflection remains constant after the first three or four reverberations. At 120 kbar, it reaches a peak and then decays with a time constant of about 280 ns. Transmission experiments show a shift of absorption edges towards the red at an initial shock pressure of ~ 5 kbar by amounts which cannot be explained simply as thermally enhanced excitation of normal vibrational states. At a pressure of < 20 kbar, extinction through the range 2,500-4,000 Å is complete. Transmission in the visible is almost completely extinguished at 120 kbar in this reverberation geometry.

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A schematic diagram of the transmission experiment is shown in Fig. 1. The projectile is 10.16 cm in diameter and 20.32 cm long, driven by high-pressure gas13. The reflection experiment differs from Fig. 1 in that the flash lamp is replaced by a cavity behind the impactor and the left face of the sapphire impactor is coated with a light absorber to minimize two-way transmission through the cell. When two-way transmission is desired the left face can be made a mirror. With the reflector in this position, it is unshocked during the significant part of the experiment.

A transmission record is shown in Fig. 2. The region 3,580-2,900 Å is an absorption band; there is another below 2,680 Å, but its edge is obscured by decreasing sensitivity of film and grating. At impact the reflection from air-sapphire interfaces between target and impactor is reduced, causing the barely perceptible increase in density across the film at a. At b, the shock initiated at impact has traversed the 2 mm face of the cell and entered the CS2. At c, the shock in CS2 has traversed the cell for the first time and reflected from the inside face of the back sapphire plate. The strength of the first shock is ~5 kbar. On reflection this increases to 12 kbar, which is apparently enough to cause complete extinction of the transmitted light in the lower transmitting region. Temperatures for these first two shocks were calculated to be 398K and 482K respectively; the apparent change in the absorption edge which is initially below 2,670 Å is > 0.23 eV; for the upper edge at 3,580 Å the change is 0.11 eV for the first shock. The shift due to the second shock in the upper edge is ~ 0.12 eV. Transmission in the visible (not shown) was little affected at the final pressure of ~ 55 kbar.

The record from a reflection experiment is shown in Fig. 3. In this experiment the left-hand face of the sapphire impactor, C in Fig. 1, is silvered, there is no flash lamp, and the record to the left of b in Fig. 3 represents two-way transmission through the CS₂ sample. The first shock, with amplitude of 8 kbar, enters the CS₂ at point b and completes its traverse of the CS₂ at c. The extinction of light during this interval is similar to that recorded in Fig. 2, but pressure of the first shock is larger. ~8 kbar, and calculated temperature is 427K. Reflection of the shock at the second CS2 sapphire interface at c initiates

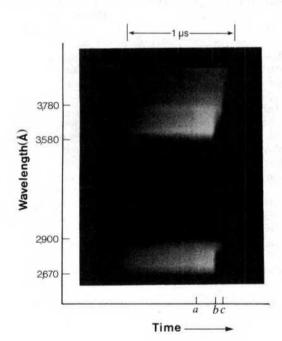


Fig. 2 Time-resolved spectrogram of light transmission through the CS2 filled cell shown in Fig. 1. Light regions represent transmission. a, impact occurs; b, shock arrival at sapphire-CS2 interface; c, second shock traversal of CS2 cell (first reverberation). CS_2 sample is 145 μm thick and ~ 3 cm in diameter. Time resolution is ~30 ns; spectral resolution is ~30 Å. Final pressure is 55 kbar. Shot number 81-010. ×3.2.

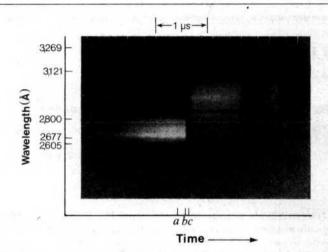


Fig. 3 Time-resolved spectrogram of two-way transmission through and relection from a CS2-filled cell. a, Impact occurs; b, shock reaches front interface of CS2-sapphire; c, shock completes first traverse of CS2 cell. Cell thickness is 69 µm. Time and spectral resolutions as in Fig. 2. Final pressure is 80 kbar. Shot number 80-010, ×1.6.

the optical reflection process. The strength of the reflection in the next few reverberations builds up to a value which remains constant until the shock reaches the rear face of the second sapphire. This is corroborated by a photomultiplier record (not shown) of the total light intensity plotted against time, integrated over the wavelengths indicated in Fig. 3. Final temperature at 80 kbar is ~870K.

Measurements of resistivity of the CS2 in these shock conditions give values of the order of $10^{+4} \Omega m$, which is too large to explain either the observed extinction or reflection. Transmission spectra in the visible show that transmission for wavelengths ≥4,000 Å persists at a final (ring-up) pressure of 55 kbar. This confirms qualitative experiments reported elsewhere 14.

Final pressure in these experiments is reached by a series of reverberations, so they are not directly comparable to single shock experiments to the same pressure. Final temperature is less in the reverberation cell than in a single shock.

These experiments should allow processes of molecular interaction which have heretofore been inferred from indirect evidence to be observed directly and open new avenues for studying the dynamics of chemical processes in highly compressed matter. Further details can be found in ref. 15.

We acknowledge the continuing support of this research by the United States Office of Naval Research under contract no. N00014-77-C-0232 and the encouragement provided by Dr Richard S. Miller.

Received 15 December 1981; accepted 29 February 1982.

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Lipids in Harmattan aerosols of Nigeria

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Terrestrial lipid detritus has been found in some pelagic sediments at great distances from land (for example, in the North Atlantic) and aeolian transport has been invoked as one input mechanism to explain this observation¹. This is supported by the presence of the same lipids in oceanic aerosols, presumably derived from major wind systems originating on land^{2,3}. Thus, it is vital to examine whether this lipid signature is observed on the continent in the ambient aerosols towards their source regions (such as the Harmattan). We have analysed solvent-extractable lipids in Harmattan aerosols collected in Nigeria and report here that the distributions of hydrocarbons indicate both a major contribution of plant waxes and lesser components of a complex composition, believed to originate from algal and other microbiological detritus in desiccated lacustrine muds or from eroded, recycled sedimentary organic matter.

The origins, nature and fate of Saharan dusts have been discussed elsewhere⁴. Organic material in dust samples collected off the coast of north-west and south-west Africa has been analysed^{2,3} but most of the samples are believed to have a different source from the Harmattan dusts. The Harmattan aerosols are believed to arise in the Faya Largeau area of the Chad Basin⁵ and are carried by the prevailing winds over Nigeria and other parts of West Africa to the Atlantic Ocean (Fig. 1). Analysis of organic matter in dusts collected off the west African coast has indicated a high concentration of organic detritus³. For a better understanding of the nature and origin of the organic matter, it was desirable to sample the dust aerosols closer to their presumed origin. High-volume air samplers with quartz fibre filters capable of collecting particulates down to ~1 µm diameter were used6. Samples were collected in varying conditions of visibility at several locations in Nigeria (Fig. 1) over two Harmattan seasons. A major problem in organic analysis of aerosols is distinguishing between natural and anthropogenic materials. Anthropogenic components could be present in the local atmosphere and would originate from emissions by vehicle exhausts, kerosene lamps and stoves.

The locations, sampling dates and analytical data for the samples that we analysed are presented in Table 1. Total solvent-extractable organic matter (lipids)⁶ was 600–6,000 ng m⁻³ of air, of which 100–2,000 ng m⁻³ consisted of hydrocarbons and the total loadings of aerosol were typically 300–900 µg m⁻³ (Table 1). Data are shown for samples collected on typically dusty days at a site in Jos (samples 1–4). This site was chosen to minimize both the effects of local soil dust (elevation 10 m above ground) and anthropogenic interferences (outside the town and upwind of the prevailing northeasterly winds). Further samples were taken in Jos (number 6) and downwind from Jos (number 12). The samples from Sokoto and Kano were taken upwind from the cities and at Maiduguri in the south-west sector (wind from north-east). The samples from Ibadan were taken within the city.

Examples of typical gas chromatographic-mass spectrometric (GC-MS) total ion current traces, equivalent to gas chromatograms, for total hydrocarbon fractions are shown in Fig. 2. An

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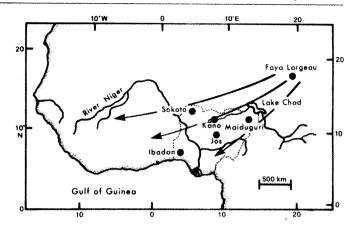


Fig. 1 Location map of the sampling sites and Harmattan wind in West Africa.

outstanding feature of sample 1, upwind from Jos is the value of the carbon preference index (CPI)^{6,7} of 7.8 for the higher n-alkanes exhibiting the typical plant wax predominance of odd carbon number alkanes and maximum at n-C29 (ref. 8). For comparison, the typical CPI range for various plant waxes is ≥5 (refs 8, 9). Also prominent is a complex unresolved mixture (hump) of hydrocarbons centred at the relative retention time of carbon number approximately 19.5. These narrow humps are typically present in degraded detritus of algae and other microorganisms¹⁰⁻¹⁵ and could have such an origin here. An alterative source may be a recycled component of hydrocarbons (both hump and alkanes) eroded from sedimentary outcrops in the regions where the aerosols are generated. Such recycled material has, for example, been reported in recent sediments in a Swiss lake16. An unambiguous origin for the narrow hump in these aerosols can therefore not be assigned, based solely on hydrocarbon data. The isoprenoid alkanes 2,6,10,14tetramethylpentadecane (pristane) and 2,6,10,14-tetramethylhexadecane (phytane) are also present as minor components. The ratio of hump to n-alkanes is low (1.4), confirming a relatively low contribution of anthropogenic organic matter, which would be expected to contribute a high proportion of aromatic and unresolvable materials. Based on GC-MS analyses, the amount of aromatic compounds in this sample is very low. This type of compound distribution is observed for samples 1-4 (Table 1).

Data are also shown (Table 1, samples 5 and 6) for aerosol samples taken on moderately clear days in town and in southwest Jos (at the town limit). These show values for alkane CPI intermediate and ratio of hump to normals equal to those of the samples taken upwind of Jos compared with that taken at Ibadan. This organic matter is probably a mixture of natural and petroleum-derived materials.

In contrast, an aerosol sample collected on a day of moderate visibility in the large city of Ibadan (sample 10) shows a high contribution of petroleum residues. This is indicated by a CPI value of 1.7 and the appearance of a new hump of hydrocarbons centred at the relative retention time of carbon number approximately 28.5 (Table 1, Fig. 2b). The ratio of hump to n-alkanes is high (4.3). CPI values typical for petroleum are ~ 1.0 and ratios of unresolved (hump) to resolved components are high 6.17 The petroleum origin has been further confirmed by GC-MS analyses, which demonstrated the presence of the characteristic molecular markers⁶. Gas chromatograms of hydrocarbon fractions of vehicle exhaust emissions show unresolved humps centred at the relative retention time of the carbon number range C₂₄-C₂₈ (refs 6, 9, and B.R.T.S. unpublished data). This type of contamination is also observed for samples 5 and 6 in Jos and Maiduguri (number 9, Table 1).

Normal fatty acids are present in all samples (80-1,000 ng m⁻³) and exhibit a high even-to-odd carbon number

T' 1	T	 I m m m l m m l m m l	Jaka Can	T Y a more a éta a	aerosols of Nigeria

	ample	Lat.	Altitude	Date	Visibility	Volume sampled	Total extract	Total HC	Hump ÷	Hump max. at		√ax. at
No.	Location	Long.	(m)	collected	(∼km)	(\mathbf{m}^3)	(ng m ⁻³)	(ng m ⁻³)	normal	C no.	$(C_{25}-C_{34})$	C no.†
1	Jos 3 (upwind)	9°56′ N 8°54′ E	1,200	9 March 1979	2	720	2,200	1,400	1.4	19.5	7.8	29
2	Jos 5 (upwind)	9°56′ N 8°54′ E	1,200	12 March 1979	8	780	660	240	1.6	24	5.9	29
3	Jos 8 (upwind)	9°56′ N 8°54′ E	1,200	7 December 1979	2.5	720	1,700	110	2.0	20.5	3.7	29
4	Jos 11 (upwind)	9°56′ N 8°54′ E	1,200	4 February 1980	1	930	2,400	150	1.4	20	5.7	29
5	Jos 6 (in town)	9°56′ N 8°54′ E	1,200	22 March 1979	8	670	6,600	2,600	7.7	28.5	3.3	29
6	Jos 12 (downwind)	9°56′ N 8°54′ E	1,200	14 February 1980	4	1,740	3,300	820	4.0	28.5	2.4 (1.5) (6.7)	29
7	Kano	12°00′ N 8°30′ E	520	5 February 1980	1	~600	~6,000	~1,700	2.6	28.5	3.0	29
8	Sokoto	13°03′ N 5°14′ E	290	22 February 1980	8	1,610	1,200	210	4.0	28.5		29, 31
9	Maiduguri	11°49′ N 13°09′ E	290	30 January 1980	8	2,650	5,700	1,400	3.9	29		29, 31
10	Ibadan	7°23′ N 3°53′ E	220	25 January 1980	8	3,640	6,000	840	4.6	28.5	1.7	29

Carbon preference index, summed from C25 to C34, odd divided by even,

predominance (CPI = 4-11) with maxima at C_{16} , C_{26} and C_{28} , typical of plant waxes and other biogenic sources¹⁸. Minor amounts of anteiso-fatty acids (C₁₃-C₁₇) and traces of iso-fatty acids are part of the fraction. They are commonly found in microbial lipids¹⁹, further supporting a possible recent biological origin for the hump in the hydrocarbon fraction. Normal fatty alcohols are also present in all samples (230-3,000 ng m⁻³) and exhibit a high even-to-odd carbon number predominance (CPI = 4-11) and maxima at C_{28} and/or C_{30} , again typical of plant waxes and other minor biogenic sources¹⁸. These compounds are not found in petroleum with these distributions and concentrations.

We believe that the samples of Harmattan aerosols collected upwind of Jos, Kano and Sokoto contain largely natural organic matter carried with the dusts or adsorbed on the particulates. The lipid material is composed of primarily plant waxes and secondary compounds derived from degraded microbiological detritus (probably mainly of algae) and/or possibly from eroded sedimentary organic matter. In a study of Harmattan dust

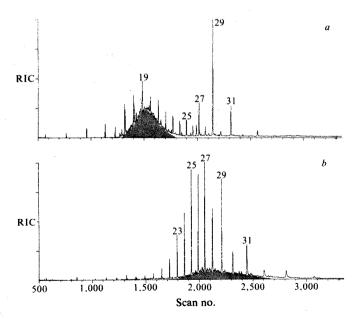


Fig. 2 Examples of total ion current traces from the GC-MS analyses of hydrocarbon fractions. (Numbers over the peaks refer to their respective carbon number.) a, Jos, upwind and b, Ibadan, urban.

deposition in Nigeria, remobilization of existing dust has been invoked to explain a lack of correlation of dust deposition which visibility and solar radiation in the latter half of the season²⁰. Our samples were collected mainly during the latter half of the dust seasons. The lipid material in our samples may arise predominantly in the Sahara like the dust itself. An alternative and more likely explanation is that the lipids originate predominantly in more fertile areas of Chad and Nigeria downwind of the source. Plant wax lipids could be released by a sand blasting effect of the dust on plant surfaces, while the minor microbial detritus could arise by the remobilization of dust from dried-up wadis and lakes, with a possible eroded sedimentary component.

Further such analyses will permit the tracking of aerosol parcels by their lipid signature back to their respective source regions, and will complement the corresponding mineralogical characteristics.

We thank Dr. C. Ekweozor for assistance with sampling in Ibadan, Dr Leon C. Jacobson for assistance with sampling in Maiduguri and Mr E. Ruth for GC-MS data. We also thank the NSF, Atmospheric Research Section (grant no. ATM 79-08645) for financial support.

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[†] Dominant homologue is underlined.

Temperature of egg incubation determines sex in Alligator mississippiensis

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The factors controlling sexual differentiation in crocodilians are unknown, but heteromorphic sex chromosomes are absent from all species1. Nichols and Chabreck2 speculated that the sex of Alligator mississippiensis was not rigidly determined at the time of hatching but could be influenced by the post-hatching environment. They presented little evidence to support their hypothesis3 (no histological sections of hatchling gonads, no indication of the sex ratio at hatching), and their study failed to take account of habitat preferences of adult male and female alligators4. Here we demonstrate by laboratory and field experiments, that in A. mississippiensis: (1) Sex is fully determined at the time of hatching and naturally irreversible thereafter, and depends on the temperature of egg incubation, temperatures ≤30 °C producing all females, ≥34 °C yielding all males. (2) The temperature-sensitive period is between 7 and 21 days of incubation. (3) Natural nests constructed on levées are hotter (34 °C) than those constructed on wet marsh (30 °C), thus the former hatch males and the latter females. (4) The natural sex ratio at hatching is five females to 1 male. (5) Females hatched from eggs incubated at 30 °C weigh significantly more than males hatched from eggs incubated at 34 °C. This weight difference constitutes a possible selective evolutionary advantage of temperature-dependent sex determination (TSD) in alligators in that females become large and sexually mature as early as possible. The occurrence of TSD in alligators has wide-ranging implications for embryological, teratological, molecular, evolutionary, conservation and farming studies as well as for theories relating to the extinction of other Archosaurs.

Laboratory studies⁵⁻¹⁰ have indicated that in five families of turtles and two of lizards, the temperature of egg incubation affects the sex ratio of hatchlings. Only one study^{5,6} has investigated the effects of natural nest temperatures on sex determination in turtles, and even this involved the construction of artificial nests. There has been no demonstration of a selective evolutionary advantage for the occurrence of TSD in reptiles^{5,6}. Theoretically, such putative selective advantages could be considered within the framework of the Charnov-Bull model^{5,6,11,12}, which has not been experimentally confirmed in higher vertebrates. This model suggests that there is an advantage in allowing the sex of an embryo to respond to its immediate environment, that is, that hatchlings from a cold nest could become either good females or sub-standard males (or vice versa) and that the opposite is true for warm nests.

Alligator eggs (500) were removed from wild nests (Fig. 1) in the Louisiana swamps within 12 hours of egg laying. Only banded³ eggs, laid by middle-aged females, were used in laboratory experiments. These eggs have high fertility, a low spontaneous malformation rate and are homogeneous with respect to egg length, width and initial weight³ (see Table 2). Eggs were randomly placed in one of six groups and incubated (in nesting media³) at 26, 28, 30, 32, 34 or 36 °C, in water-jacketed, air circulating incubators at 100% humidity (Table 1). Eggs incubated below 26 °C or above 36 °C died. After 60 days incubation

(the normal incubation period is 65 days), all eggs were killed and the enclosed alligators fixed in 10% formal saline. Using a dissecting microscope, we noted the presence or absence of oviducts or vas deferens, and the surface appearance of the gonads. The entire reproductive systems of all animals were excised, serially sectioned (8 μm) in the transverse, sagittal and horizontal planes and stained with haematoxylin and eosin, periodic acid Schiff and haematoxylin, Mallory and Weigert Van Gieson. Hatchling gonads were histologically differentiated into ovaries or testes and showed no evidence of bisexuality or hermaphroditism. Sex is evidently fully determined by the time of hatching. Moreover, alligators raised for 1 year in a variety of conditions showed no signs of sex reversal and no evidence of bisexuality. In all experiments, sex was determined macroscopically as well as by serial histological sectioning.

All eggs incubated at ≤30 °C developed into females, whereas all those incubated at ≥34 °C developed into males (Table 1); eggs incubated at 32 °C had a sex ratio of 86.7%:13.3% females to males. There was no significant difference in embryonic mortality between eggs incubated at 28 °C, 30 °C, 32 °C or 34 °C (Table 1). Even if all the dead embryos had been of opposite sex to those that were living,

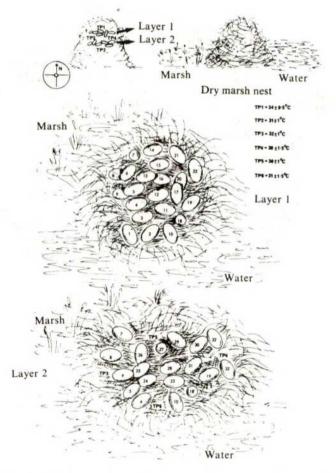


Fig. 1 Map showing the location of eggs and temperature probes TP1-TP6 in a dry marsh nest of A. mississippiensis. The nest consists of a mound of vegetation (mostly Spartina patens) and is ~4 ft in diameter and 3 ft high. Average temperature recorded by TP1 was 34 ± 0.5 °C, and for TP2-TP6, 31 ± 1 °C. Eggs 12, 13 and 16 contained male alligators (average intact weight of hatchlings = 37.5 ± 0.5 g, average weight of hatchling minus yolk = 28.8 ± 0.3 g, average weight of yolks = 8.5 ± 0.5 g); eggs 4-6, 8, 9, 14, 15, 20, 21, 25 and 27-31 contained female alligators (average intact weight of hatchlings = 39.1 ± 2.8 g, average weight of hatchling minus yolk = 28.7 ± 0.5 g, average weight of hatchling minus yolk = 28.7 ± 4.4 g, average weight of hatchling minus yolk = 28.7 ± 4.4 g, average weight of yolk = 10.7 ± 1.4 g); and eggs 1-3, 7, 10, 11, 17-19, 22-24, 26 and 32 were infertile, dead or malformed. The small size of these eggs indicated that they had been laid by a young female³.

Table 1 Sex and mortality of alligators removed after 60 days from eggs artificially incubated at varying temperatures

			Temperature of egg	incubation (±0.2 °C)	4 . 58.454
	26 °C	28 °C	30 °C	32 °C	34 °C	36 °C
No. of eggs No. of dead embryos (% of total)	50 40 (80)	100 4 (4)	100 3 (3)	100 2 (2)	100 6 (6)	50 43 (86)
Females % Of total eggs % Of living eggs	20* 100*	96* 100*	97 100	85 86.7	0 0	0
Males % Of total eggs % Of living eggs	0	0	0	13 13.3	94 100	14 100

All eggs were recovered from wild nests within 10 h of laying, and all were laid by middle-aged female alligators3. Infertile eggs were excluded and the average

egg weights at the start of incubation were statistically similar in all temperature groups (Table 2).

* These animals were developmentally retarded and still exhibited yolk external to the body wall. They were at a stage of embryonic development equivalent to day 48 embryos incubated at 32 °C. Thus they were excluded from the weighing analyses in Table 2.

the differences between the temperature groups would still be highly significant (Table 1). Thus, the temperature of egg incubation determines the sex of A. mississippiensis in laboratory experiments.

To determine the temperature-sensitive period during egg incubation critical for sex determination, eight groups each consisting of 20 eggs were incubated first at 30 °C and then switched to 34 °C after 1, 2, 3, 4, 5, 6, 7 and 8 weeks respectively. A comparable series commenced incubation at 34 °C and were switched to 30 °C. We found that sex was determined by the incubation temperature during weeks 2 and 3; thereafter changes in incubation temperature had no effect. It was easier to produce females, particularly during week 3 of switches from 30 °C to 34 °C. The temperature-sensitive period for alligators occurs much earlier than for other reptiles⁵⁻¹⁰, but alligator eggs are laid at an advanced embryonic stage³, so that the embryonic stages during which sex is determined are comparable. Again there was no evidence of sex reversal, rather the embryos showed progressive gonadal differentiation and were committed to a particular phenotype after the temperaturesensitive period.

Although we have shown that constant incubation temperatures determine sex in laboratory experiments, they may not do so in nature where nest temperatures fluctuate depending on the geographical location of the nest (levée, wet or dry marsh, shaded, exposed), the prevailing climate and whether embryos have a genetic disposition towards one sex^{5,6}. To investigate the role of temperature on sex determination in nature, we placed temperature probes (Taylor Instruments) connected to continuous chart recorders, in different locations (top, bottom and four sides of the egg cavity) within six typical wild alligator nests (see Fig. 1). Two nests were on dry levées, two on wet marsh and two on dry marsh. The natural nest construction was not disturbed during insertion of the probes. Temperatures were recorded throughout the incubation period. After 60 days of incubation, nests were opened, the eggs counted and an accurate nest map drawn, to indicate the positions of the eggs and temperature probes (Fig. 1). All eggs were opened and the alligators coded, fixed and sexed. Sex of the animal was correlated with position in the nest and nest temperature. The average temperature of dry levée nests was 34 ± 0.7 °C at the periphery and bottom of the nests (TP2-TP6, Fig. 1) and 35 ± 1 °C at the top centre of the nests (TP1, Fig. 1): 99% of living eggs (n = 59) were males. The average temperature of wet marsh nests was 29 ± 1 °C at the periphery and bottom of the nests and 30 ± 1 °C at the top centre of the nests: all living eggs (n = 61) were females. The average temperature of dry marsh nests was 31 ± 1 °C at the periphery and bottom of the nests and 34 ± 1 °C at the top centre (Fig. 1). Males hatched from eggs at the top centre of these nests and females from eggs at the periphery and bottom of the nests (Fig. 1). The average sex ratio for dry marsh nests was 5:1, females to males (n = 63). Evidently, sex is determined by egg incubation temperatures in nature as well as in laboratory experiments.

It has been argued^{5,6} that genotypic and environmental sex determination could co-exist if animals had a weak sex-determining locus which operated in most environmental conditions, but which was overridden in extreme conditions. Predictions from Bull's model^{5,6} show that if genotype and temperature both determine sex, then a sex ratio of 1:1 should exist in the hatchling population (but not necessarily in the adult population where differential sexual mortality may occur). To determine the sex ratio at hatching in a wild alligator population, we collected eggs (8,000) from all alligator nests laid within a large area (~2,000 acres) of typical habitat (including levées and marsh) in the Rockefeller Wildlife Refuge, Louisiana. These eggs were collected after 5 weeks of incubation, after the temperature-sensitive period and before maximum egg predation by racoons⁴. They were artificially incubated in nesting media³ at 32 °C, allowed to hatch, raised in controlled environmental chambers and sexed externally at 1.5 yr old (we did not feel justified in killing 8,000 alligator hatchlings for microscopic sexing). As the sex ratio at hatching may vary from year to year, depending on climatic conditions, the above protocol was performed during 1978-81. The average sex ratio for the 4 years was $5 \pm 0.7:1$, females to males. This is consistent with field observations (marsh nests are more numerous than levée nests) and with laboratory experiments, in which viable females are produced over a greater temperature range (28-32 °C) than males (34 °C). This heavily biased female sex ratio means that classical genotypic mechanisms probably have no role in alligator sex determination^{5,6}; but it also presents a problem for sex ratio theory. Fisher¹³ noted that selection within random-mating populations favours a primary sex ratio of 1:1, provided sons and daughters are equally costly to produce. Such a 1:1 result does not necessarily apply to TSD¹⁴, but even using Bull's theory, it is difficult to account for the extreme sex ratio observed in alligators. Therefore, either the present sex ratio is a recent development, perhaps resulting from changes in the availability of various nest sites, or some property of population structure or inheritance is peculiar and selects for the observed sex ratio.

We investigated possible selective advantages for the evolution of TSD in alligators. During incubation, yolk and albumen are progressively used for embryonic growth and metabolism¹ Alligators hatch with a considerable volume of absorbed abdominal yolk which serves as a food source for the posthatching period^{3,4}. As the rate of metabolism of poikilotherms, for example, alligators, depends on temperature 16, we investigated whether there was any weight difference between male and female hatchlings incubated at 34, 32 or 30 °C both in the laboratory (Table 2) and in the field (Fig. 1). Since the size of any hatchling is related to the freshly laid egg weight³, the latter was controlled so that there were no significant differences between the temperature groups at the start of the experiment (Tables 1, 2). It was impossible, however, to control freshly laid egg weight in the nest map field experiments (Fig. 1), as this would have meant disrupting the nests. In laboratory experiments females hatched at 30 °C weighed significantly more than males hatched at 34 °C (Table 2). This weight difference is due to the females having significantly more absorbed yolk than the males (Table 2), which is consistent with the postulated lower rate of embryonic metabolism at 30 °C. As all eggs were incubated at 100% humidity, the latter is unlikely

Table 2 Statistical analysis of the weights of day 60 alligator embryos (hatchlings) and their absorbed yolks as a function of egg incubation temperature and sex

Temperature of egg incubation (±0 2 °C)	No. of eggs	Sex of hatchling	Initial weigh		Weight of hatchl		Weight of minus y		weight o	f yolk (g)
30 °C 34 °C	97 94	Female Male	65.1 ± 5.7 66 2±6 1	P>02	47 6±3.1 43 7±4 0	P<0.001	37 8±3.9 38.2±4.6	P > 0.5	8 1±2.2 5.3±1 9	P < 0.001
32 ℃ 32 ℃	85 13	Female Male	65.8±68		46 2±4 5 45.8±3 9	P>0.7	36.8±4.2 37 0±4 0	P>0.8	7.7 ± 2.5 7.6 ± 2.8	P>09

The initial egg weights were similar in all temperature groups, although in the 32 °C group it was clearly impossible to determine at the onset of the experiment which eggs would become males and which females. The hatchlings were fixed and weighed intact (minus extra-embryonic membranes) to the nearest 0 2 g. The absorbed yolk was then carefully dissected out of the abdomen and both it and the devolked hatchling weighed separately, to the nearest 0.2 g Frequently the sum of the last two weights did not correspond exactly to the first weight due to very small losses of blood. Statistical comparison of any two means was calculated using the t-test18 Values are the mean ± s d.

Table 3 Length and weight of 1-yr-old female and male alligators hatched from eggs incubated at 30 °C and 34 °C respectively

Sex	No of animals	Total len	gth (cm)	Weigh	t (g)
Female	12	63.25 ± 2.54	·	708 3±78.5	
Male	ncubation) 4 ncubation)	53 34±4 83		475±108.5	P<0 001

The alligators were reared after hatching in artificial environmental chambers in identical conditions of, for example, temperature, food, light, water, exercise Values shown are the mean ± s.d. The means for the two groups were compared using Student's t-test18. Total length was measured from the snout tip to the end

to be important¹⁵, although decreased humidity could be associated with increased nest temperatures in the wild. Similar trends were observed in the field—levée nests produced lighter males, wet marsh nests heavier females. Moreover, in a single dry marsh nest, where the initial egg weights were similar, males hatched significantly lighter than females (Fig. 1).

The sexual difference in hatchling size and weight was maintained during the first year of life in alligators raised in environmental chambers4 in identical post-hatching conditions (Table 3). However, once alligators reach a length of ~1 m (at 2-3 yr old) the growth rate of females declines faster than that of males¹⁷. Thereafter, males grow faster and for a longer time than females¹⁷, probably as a result of their differing endocrinology. Sexual maturity in alligators depends on size, both sexes maturing when ~1.8 m long. Because of the initial heavy hatchling weight, and resultant rapid early growth, females become sexually mature either before, or at the same age, as males. The number of years of functional reproductive activity in females is about one-half that of males³. In addition, over 50% of the eggs laid by small (young) females are either infertile or malformed³, while social order favours breeding of larger females before smaller ones. These data, combined with the fact that one male can fertilize several females in any breeding season, make it highly desirable that female alligators should become large and sexually mature as quickly as possible. We suggest that this is a possible selective advantage for TSD in alligators. Thus, animals incubated at the low temperatures which produce females hatch with more energy reserves (absorbed yolk), develop, grow and become sexually mature faster than those incubated at the higher temperatures, which yield males (Tables 2, 3). If genotypic mechanisms determined sex, then the latter would be unrelated to incubation temperature and thus hatchling weight, so that 50% of the offspring would be light females. These animals would mature more slowly, enter the breeding population much later and contribute fewer offspring per lifetime than their heavier counterparts. In terms of the Charnov-Bull model^{11,12}, if the hatchling is light, there is a selective advantage in it being an average male, rather than a below average female. Temperature-dependent sex determination allows expression of this advantage by closely associating hatchling weight with sex. Depending on the life history of other reptiles, there may be selective advantages in being either a light or heavy female or male which could explain why low

temperatures produce females in lizards and males in turtles⁵⁻¹⁰. Interestingly, if alligator eggs are incubated at 32 °C there is no difference in the weights of the resulting male and female hatchlings; both follow the heavier 30 °C female pattern (Table 2). Perhaps the heavy males hatching at this temperature become the large males in the adult population.

Male and female alligators occupy different micro-habitats after hatching: males inhabit open water canals beside levées and females the marsh⁴. Additional selective advantages, for example in terms of predation, may accrue from being a larger, faster growing female in the marsh or a smaller male in the open water. Interestingly, the habitat of adult male and female alligators is closely related to the geographical location of maleand female-producing nests (the former on levées beside open canals, the latter in the marsh). This may represent another selective advantage for TSD because neither male nor female hatchlings have to move great distances (when they would be subject to predation) from 'unsuitable' nesting sites to the preferred adult habitat.

By exploiting TSD to control the sex of alligator embryos experimentally, it is possible to study sexual differences in early embryogenesis as well as differences in the metabolism and action of teratogens³. In addition, TSD has wide implications for habitat planning in conservation studies and for pen design, egg collection times and artificial incubation temperatures in alligator farming. Moreover, if other ancient Archosaurs, for example, dinosaurs, also showed TSD with perhaps a different male-female threshold temperature, then this may explain the selective extinction of these groups in response to a relatively sudden, continuous change in climate to one that is either hotter or colder³.

We thank L. McNease and staff of the Rockefeller Wildlife Refuge for assistance with egg collection, J. Bull for valuable discussion and N. Steinberger for access to preliminary data, obtained by the late C. L. Yntema, which confirm our findings. This work was supported by MRC Grants G979/386/C and 8113610CB, grant EP109/74/75 from the Northern Ireland Eastern Health & Social Services Board, a research travel grant from the Wellcome Trust of Great Britain, a Dixon Research Scholarship from the Queen's University of Belfast, and a Churchill Fellowship, all awarded to M.W.J.F.

Received 16 October 1981, accepted 16 March 1982

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Effect of adh genotype and heat stress on alcohol tolerance in Drosophila melanogaster

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The alcohol dehydrogenase locus (adh) is polymorphic in most natural populations of Drosophila melanogaster1-11. Eight alleles have been identified on the basis of allozyme electrophoretic mobility and thermostability; two of these, adh Fm and adh Sm , generally have combined frequencies of $\geq 90\%$. In four widely separate locations these major alleles display a clinal change in frequency, that of adh Fm increasing with latitude and altitude^{3-6,11}. Biochemical studies have shown that the Fm allozyme (ADHFm) is more active, but less heat-resistant, than ADHsm(refs 12-14). The fact that these differences are associated with the geographical distribution of the alleles suggests that environmental temperature may be a selective factor in maintaining this polymorphism. We have investigated the effect of high temperature on flies having ADH allozymes with a range of thermostabilities and report here that flies which have relatively labile enzymes show reduced survival on alcoholsupplemented food compared with flies possessing more heatresistant allozymes.

The specific physiological role of ADH has been much debated, but as the results of numerous experiments indicate that alcohol tolerance is a function of overall ADH activity levels 15-20, the enzyme is thought to contribute to the flies' ability to feed and breed in fermenting fruits. The relatively low temperature at which ADH can be inactivated in vitro (40-43 °C)^{5,7,14} suggests that a similar effect could be produced in living flies encountering these temperatures, for example while feeding on fallen fruits warmed by the sun or by heat of fermentation. As ADH is necessary for survival on alcoholic media, alcohol tolerance should be reduced if a significant portion of a fly's ADH is denatured by non-lethal heat exposure. Under such heat and alcohol stress, the relative thermostabilities of different allozymes may have adaptive significance.

In designing an experiment to test this hypothesis, it was desirable to eliminate, as far as possible, the effects of other loci which might alter ADH activity. We therefore used a breeding scheme designed to derive five strains, each homozygous for a different adh allele, but with nearly uniform genetic backgrounds. By using laboratory stocks with various visible mutations and cross-over suppressors, strains were developed which differ only in a small region (about six map units long) surrounding the adh locus (II, 50.1). The strains all have an unmarked X chromosome derived from the Cy/Pm, D/Sb marker stock (stock 97; Amherst, Massachusetts); second chromosomes in which both ends (from 0 to 48.5⁺ and from 51.5⁺ to 108) came from marker stock P232 (Pasadena, California); and third chromosomes obtained from a male caught in the wild. The fourth chromosome was uncontrolled. A sixth stock was not involved in the breeding scheme; this stock was obtained from Davis, California (stock R 72) and carries adh F

(strain 1 in Fig. 1). Allozyme and allele symbols are based on the electrophoretic mobility and thermostability of the enzyme: Fr, fast mobility, heat-resistant; Fm, fast mobility, moderately heat-sensitive; Fs, fast mobility, heat-sensitive; Sm, slow mobility, moderately heat-resistant; Ss, slow mobility, heat-sensitive.

Male flies from uncrowded culture vials were collected soon after eclosion and aged for 5 days on unsupplemented instant Drosophila medium at 23 °C. For the heat treatments, flies were placed in empty, cotton-stoppered shell vials containing a piece of moist blotting paper to maintain adequate humidity. Vials were heated for 13 min in a 40 °C water bath. The flies were then allowed to recover in the same vials for ~4 h by which time they were standing or walking around. The treatment period of 13 min was chosen as the longest time for which almost all flies could survive. It was observed that very small flies usually did not recover consciousness. This occurred sporadically in all the strains and did not seem to be genotypespecific. After the recovery period, flies were transferred to vials of instant medium supplemented with various concentrations (by volume) of ethanol. After 2 days the number of live flies was counted to determine percentage survival. Control flies were treated in the same way, but were not heated. Figure 1 compares the survival of heated with untreated flies of each strain; differences between strains are apparent.

The alcohol tolerance of the heat-treated flies was also compared with that of the control flies for each of the six strains by examining the slopes of the regression lines obtained for each treatment (Table 1). The untreated control flies showed no difference among strains in their response to alcohol. Although there was a slight reduction in survival for both 6% and 8% alcohol, this reduction could not be related to variations in the ADH thermostabilities. By contrast, alcohol tolerance was clearly decreased after heat treatment, and the three strains having the most sensitive enzymes, ADHss and ADHss, showed the greatest reduction. That the reduced survival on ethanol was a result of a decrease in ADH activity was confirmed by direct measurements of ADH activity levels in heat-treated flies (Table 2). Spectrophotometric assays revealed that flies with ADHss and ADHss lose nearly 90% of their ADH activity when subjected to 40 °C for 13 min. Flies with ADHFm have about one-third less ADH, but as these flies had the highest initial levels of ADH activity, a substantial amount of enzyme remains effective. Finally, flies with the two most resistant allozymes, ADH^{Fr} and ADHSm, have the same levels of enzyme activity after heat exposure as before. Figure 1 shows that adh^F flies have the same alcohol tolerance regardless of heat stress, in the range of ethanol concentrations used. In the case of adhSm, there was a significant reduction in survival following heat treatments, but the slope of the regression line was not significantly different from that for the controls, indicating that survival on ethanol was not interacting with the heat treatment.

The 95% confidence intervals of the slopes of the regression lines for the heat-treated flies overlap to such an extent that the only strains that are significantly different are adh^{Fr} and adh^{Sm} , compared with the two adh^{Fs} strains. Note, however,

Table 1 Slopes of regression lines for each strain and treatment in Fig. 1

	C	Control	Heat-treated				
		Confidence	95% Confidence				
Strain	Slope	interval	Slope	interval			
adh ^{Fr}	-0.45	-1.48-0.58	-0.35	-1.43 - 0.73			
adh Sm	-1.50	-5.00- 2.00	-2.05	-4.25-0.15			
adh ^{Fm}	-1.10	-1.900.30	-3.10	-5.510.70			
adh ^{Ss}	-0.70	-1.220.18	-5.40	-10.490.31			
adh Fs	0.00		-7.50	-10.504.50			
(strain 1)							
adh ^{Fs}	-1.10	-1.600.60	-9.75	-12.337.17			
(strain 2)							

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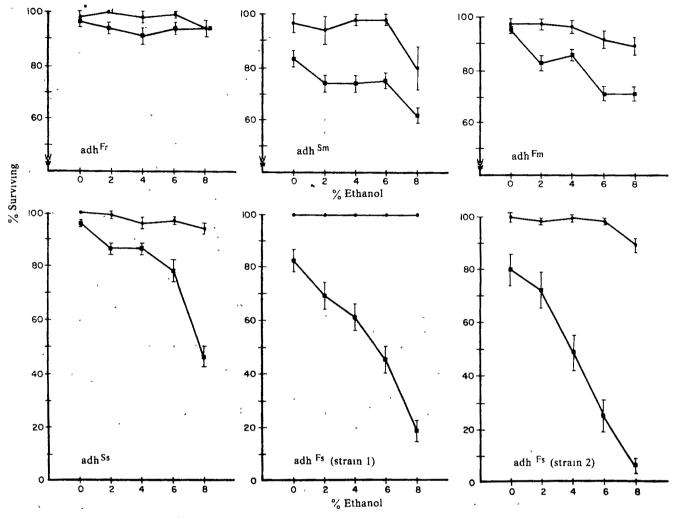


Fig. 1 Relative survival of untreated (control, •) and heat-treated (40 °C, 13 min, •) files on various concentrations of ethanol. Each data point represents the survival of 100-800 files. Bars indicate 95% confidence intervals calculated from the binomial distribution.

that if the strains are ordered by their slopes (indicating interaction of the heat and alcohol stress) the order obtained is exactly the same as the *in vitro* decrease in allozyme thermostability previously reported: Fr>Sm>Fm>Ss>Fs (ref. 21).

Our observations suggest that the variant alleles, adh^{s_i} and adh^{F_i} , may be rare in nature because they are conditionally deleterious or even lethal. Fitness of flies carrying these alleles depends on environmental temperature combined with alcohol concentration.

Other investigators have examined the survival of flies with different adh genotypes following heat shock. Johnson and Powell²² tested flies from four natural populations. In two of the samples, they observed a higher frequency of adh^{8m} among the survivors of a heat shock (40 °C for 30 min) than among a group of untreated flies. No increase in the frequency of either allele was observed in the other two samples. Milkman²³ found no difference in adh frequency between the survivors and non-survivors after heat treatment (40 °C for 10 min). It should be emphasized that Johnson and Powell did not expose their flies, either during or after the heat treatments, to any situation in which ADH is known to be essential.

In our experiments, heat-treated flies with different allozymes showed similar levels of survival in the absence of additional ethanol: $\sim 95\%$ for adh^{Fr} , adh^{Fm} and adh^{Ss} strains, and 82% for adh^{Sm} and adh^{Fs} strains. Only when the food was supplemented with at least 4% ethanol did the survival differences among the strains become significantly correlated with enzyme thermostability. Thus the relevance of Johnson and Powell's results²² to the maintenance of the adh polymorphism is not clear.

A correlation between environmental temperature and adh allele frequency has been observed for the major alleles in Spain⁹, along the eastern coast of the United States²⁴ and in southern Texas and eastern Mexico^{4,6}. The relationship between temperature and fitness however, may not be a simple one. In a more thorough analysis of the north-south clinal variation in a series of Australasian populations, Anderson²⁵ examined the relationship between adh frequency and various environmental variables that change with latitude. He found that differences

Table 2 ADH activity in heat-treated and untreated control flies

		I activity vet weight per min)	
Strain	Untreated controls	Heat-treated (40 °C, 13 min)	% Reduction
adh ^{Fr} adh ^{Fm} adh ^{Fs}	16.6 (0.3) 29.8 (1.3) 19.4 (1.0)	17.1 (0.4) 20.2 (0.1) 2.6 (0.4)	None 32 87
adh Sm adh St	9.8 (0.1) 12.3 (1.5)	10.0 (0.2) 1.5 (0.2)	None 88

ADH activity was measured at 25 °C in a 2 ml reaction mixture containing 2.7 M ethanol and 1 mM NAD in 100 mM Tris-HCl buffer pH 8.6, plus 0.2 ml of crude extract. The extracts were prepared by homogenizing 30 male flies in 1 ml of Tris buffer and centrifuging at 10,000 r.p.m. for 30 min. One unit of ADH activity represents an increase in absorbance of 0.001 at 340 nm. Activities represent the average for two separate groups of flies; standard deviations are shown in parentheses.

in rainfall accounted for 76% of the variation in allele frequency. None of the other variables, including several temperature statistics, could explain the remainder of the variation. He then suggested that temperature and humidity could influence indirectly the adh polymorphism through their effects on such environmental factors as competitor and predator species, types of vegetation available as food sources, varieties of decay-causing bacteria and yeasts, and rates of fermentation and evaporation of metabolic products including ethanol.

Received 16 November 1981; accepted 8 March 1982.

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It is almost certain that selection acting on the adh locus will involve a complex set of factors and mechanisms. Our results indicate that temperature can, in appropriate conditions, act directly on fitness through the inactivation of heat-sensitive enzymes.

This research was supported by NSF grant DEB 79-11538 and NIH grant RR-08043-10. Some equipment was purchased through NIH training grant T32-GM07091. We thank R. Milkman and G. Eertmoed for reading the manuscript and W. Prigge for assistance with graphics.

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Signal processing times in bacterial chemotaxis

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The bacterium Escherichia coli responds to changes in the concentrations of various chemicals in its environment¹. A cell swims along a smooth trajectory (runs), moves erratically for a brief time (tumbles) and then runs again, choosing a new direction at random². If a run happens to carry the cell up a gradient of an attractant (such as aspartate, serine and certain sugars), the occupancy of the appropriate chemoreceptor increases with time^{3,4} and a signal is sent to the flagellar motors that increases their counterclockwise bias5. On the average, this extends favourable runs and the cell moves up the gradient. The receptors for aspartate and serine 6-8 are proteins found in the cytoplasmic membrane, known as methyl-accepting chemotaxis proteins^{9,10}, and are the products of the tar and tsr genes¹¹. A cell can adapt to sustained changes of receptor occupancy by carboxymethylating these proteins¹²; it is not known, however, how these proteins signal the flagellar motors or how the signal controls the direction of flagellar rotation. Products of several *che* genes involved in signalling and adaptation have been identified^{13,14}, but with the exception of a methyltransferase¹⁵ (the *cheR* product) and a demethylase¹⁶ (the cheB product), their functions are largely unknown. In an attempt to learn more about the events that trigger a chemotactic response, we have now exposed cells to rapid changes in the concentration of attractants and repellents and measured the time required for flagellar reversal. In wild-type cells and in cells containing a cheR-cheB deletion, the response latency is ~ 0.2 s. In *cheZ* mutants, it is much longer.

Cells were tethered to glass with antibodies directed against flagellar filaments, so that rotation of a cell body could be used to monitor the rotation of a flagellar motor¹⁷. Individual cells were observed with a microscope equipped with an opto-electronic device that provided a continuous readout of the cell's angular position 18,19. A micropipette containing sodium α -methyl-DL-aspartate (α -MeAsp, a non-metabolizable analogue of the attractant aspartate) was positioned with its tip a few micrometres away from the cell, and the cell was stimulated iontophoretically by the passage of current. As the addition of α-MeAsp causes a cell to spin counterclockwise⁵, the current was switched on when the cell changed its direction of rotation from counterclockwise (CCW) to clockwise (CW). We measured the interval of time from the beginning of this stimulus to the next reversal (the response latency), with a resolution of ~ 0.03 s. A typical response is shown in Fig. 1. Stimuli were given about every 30 s, and distributions of interval lengths were constructed (see Fig. 2). The experiments were repeated with different concentrations of α-MeAsp in the pipette and the results are summarized in Table 1. When the concentration in the pipette was ≤0.01 mM, the distributions of interval lengths did not differ significantly from those observed in the absence of a stimulus (Fig. 2a). When the concentration was ≥ 0.1 mM, responses occurred in ~ 0.2 s (Fig. 2b; Table 1). When the concentration was increased by a factor of 100, the mean latency decreased by less than a factor of 2 (Table 1).

To interpret these results, one needs to know the initial concentration of α -MeAsp near a cell and the time course of the change generated by the stimulus. A cell exposed to a step change in concentration of α -MeAsp spins CCW for a time (the transition time) proportional to the change in receptor occupancy. This time is ~180 s for a shift from 0 to 0.16 mM, the apparent dissociation constant of the receptor^{20,21}. The initial concentration of α -MeAsp near a cell was estimated by suddenly bringing the pipette from far away to its final position. No response was detected with ≤ 1 mM α -MeAsp in the pipette. With 10 mM α -MeAsp in the pipette, a transition time of \sim 20 s was observed, corresponding to a jump in concentration at the cell from 0 to \sim 0.008 mM. The maximum concentration change that could be generated by the stimulus was estimated by measuring the transition time after the pipette was in position and the current was switched on and left on. Steady-state concentrations determined in this way are given in the last column of Table 1. Negative ions carried out of the pipette by the current ultimately reach the cell by diffusion. For diffusion from a point source switched on at time t=0 (ref. 22), the concentration at the cell increases as $C = C_{\infty}$ ERFC $[r/(4Dt)^{1/2}]$, where C_{∞} is the steady-state concentration, ERFC is the error function complement, r is the distance between the cell and the tip of the pipette ($\sim 4 \times 10^{-4}$ cm) and D is the diffusion constant of α -MeAsp (8.9×10^{-6} cm² s⁻¹). The concentration rises rapidly, reaching one-third, one-half and twothirds of its steady-state value in 0.01, 0.02 and 0.05 s

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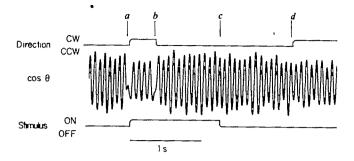


Fig. 1 A strip-chart record of iontophoretic stimulation of tethered E. coli strain AW405. The middle trace shows the cosine of the angle of orientation of the cell, $\cos \theta$; $\sin \theta$ also was recorded (not shown). These two signals were used to generate the event marker shown in the upper trace, which indicates the direction of rotation of the cell. The event marker was updated every time sin $\theta = 0$, that is, twice per cycle, and was used to trigger the stimulus. A second event marker, shown in the lower trace, was turned on when current was injected into the pipette³². The latency was determined by measuring the interval between the onset of the stimulus and the next reversal, as indicated by $\sin \theta$ or $\cos \theta$. The measurement was corrected for the lag in the electronics used to smooth these signals (0.06± 0.02 s). A spontaneous reversal occurred at a. The cell was stimulated 0.03 s after this event was flagged by the upper event marker. Another reversal (the response) occurred at b, 0.34 s later (0.28 s when corrected for lag in the electronics). The stimulus ended at c, and the cell changed direction again at d. Cells were grown as described previously²⁰ on glycerol rather than glucose, washed twice with a tethering medium containing 90 mM NaCl, 10 mM KCl, 10 mM Tris pH 7 0 and 0.1 mM tetraethylenepentamine (tetren³³), resuspended in this medium at one-tenth the original volume, sheared by passage between two syringes equipped with 26-gauge needles and washed once more Then they were tethered, as described previously2 The micropipette (F. Haer 30-31-1 Pyrex 7740 tubing pulled on a D. Kopf 700C puller) was filled with tethering medium containing, in addition, 10 mM sodium α -MeAsp (Sigma) and 0.01 mM ThCl₄ (see below). Its resistance was about 50 M Ω . Connection to the current-injection circuit was made with AgCl-coated Ag wires. The tip of the pipette was positioned 2 μm above a point 4 μm away from the point of attachment of the cell. The stimulus current was -100 nA (negative ions ejected) No positive back current was used. The bath was perfused slowly with tethering medium to prevent any long-term buildup of attractant. The cell was observed with a ×40 water-immersion phase-contrast objective (Zeiss) electrically insulated from the microscope. The experiment was done at room temperature (22±1°C). ThCl₄ was added to the medium in the pipette to neutralize negative charges on the surface of the glass³⁴ which we think were responsible for stimulus artefacts (responses that occurred without any α -MeAsp in the pipette, particularly to the passage of positive currents). As thorium is precipitated by phosphate and chelated by EDTA, these components of the standard tethering medium²⁰ were replaced by Tris and tetren respectively.

respectively. With 0.1 mM α -MeAsp in the pipette, the cells responded on the average in 0.32 s (Table 1), at which time the receptor occupancy was 2.3%. With 1 mM or 10 mM α -MeAsp in the pipette, this change in receptor occupancy should have occurred in 0.002 s or 0.004 s, respectively. The cells responded on the average 0.25 or 0.23 s later, when the changes in receptor occupancy were 10 or 69%, respectively. We conclude that the cells respond with a latency of $\sim 0.2 \text{ s}$ when the change in receptor occupancy is as small as 2% and that larger changes in receptor occupancy do not change the latency much. The latency is well within the upper limit for a chemotactic response time ($\sim 0.7 \text{ s}$) set by the rapid-mixing experiments of Macnab and Koshland³.

The proper functioning of the pipettes (see ref. 23) was checked by replacing the α -MeAsp with 10 mM fluorescein and measuring the fluorescence near the tip of the pipette with a photomultiplier mounted on a dark-field microscope. Changes in fluorescence occurred during the passage of a pulse of negative current on a time scale consistent with that predicted by the diffusion theory to within $\sim 0.01 \, \mathrm{s}$.

We looked for correlations between the mean latency of a cell and the pre-stimulus directional bias of its flagellar motor. In the 34 cells examined, the ranges of mean CW intervals, mean CCW intervals and fractions of time spent CW were 0.34-1.89 s, 0.66-3.12 s and 0.11-0.71, respectively. The mean latencies were not correlated with any of these parameters.

The following controls demonstrated that we were dealing with bona fide chemotactic responses. Wild-type cells (strain AW405) did not respond when the pipette contained only tethering medium (Table 1); they responded to benzoate (100 mM in the pipette) as a repellent, that is, by spinning CW in response to a pulse of negative current. A tar mutant (strain AW539) failed to respond to α -MeAsp but did respond to benzoate as a repellent. A tsr mutant (strain AW518) responded to both α -MeAsp and benzoate as attractants, as expected²⁴. Whenever a response occurred, the latency was \sim 0.2 s.

What steps in the signal-processing pathway cause the latency? Recall that the latency changes very little when the concentration of α -MeAsp is increased by a factor of 100 (Table 1). With large stimuli, more attractant can diffuse to the cell, enter the periplasm and bind to the receptor in a given time, so none of these processes can be limiting. Reactions whose rates are proportional to the amount of bound receptor cannot limit the latency either, because the limit is reached with changes in receptor occupancy as small as 2%. The times required for a small molecule to diffuse from the receptor to the flagellar motor or for an electrogenic impulse to travel along the cytoplasmic membrane are a few milli- or microseconds respectively, so signal transmission per se is not likely to be limiting. Finally, the time required for the flagellar motor to change the direction of rotation of a tethered cell once a reversal is initiated is 0.01 s or less²⁵, so this factor is not important. Either the tar protein activates a sequence of one or more reactions that signal the motor with a delay averaging 0.2 s, or the motor, having received the signal, is not able to initiate a reversal in a shorter time.

The latency of a strain containing a cheR-cheB deletion is normal (strain RP1273)²⁶. Therefore, excitation does not involve methylation¹², demethylation²⁶ or other processes catalysed by the cheR or cheB gene products. The latencies of strains containing cheZ mutations are very long, ~ 2 s (strains RP5006 = cheZ 292, an amber mutant, and RP5007 = cheZ 293, ref. 27). It is known from reversion analysis and interspecies complementation tests that the cheZ gene product interacts with both the demethylase (the cheB gene product) and with components of the flagellar motor involved in controlling the direction of rotation (the cheC = flaA and cheV = flaB

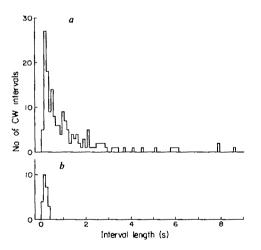


Fig. 2 Distributions of rotation intervals observed before (a) and after (b) iontophoretic stimulation of E, coll strain AW405 (as indicated in Fig. 1). Distribution a includes CW intervals observed immediately preceding each stimulus (3-5) intervals per stimulus, 171 in all, mean \pm s.d. 1.32 ± 1.93 s). Distribution b includes CW intervals observed during each stimulus (1) interval per stimulus, 24 in all, mean \pm s.d. 0.18 ± 0.10 s, corrected for the lag in the electronics). Distribution a is exponential, except for a paucity of intervals of length ≤ 0.1 s; distribution b is bell-shaped. The distribution of CCW intervals observed immediately preceding each stimulus (not shown, 179 intervals in all, mean \pm s.d. 1.36 ± 1.40 s) was similar to the corresponding distribution of CW intervals (a).

Table 1 Response of the wild-type strain to different concentrations of α -MeAsp

Concentration in	CW int	terval (s)		Total no. of	Steady-state concentration at
pipette (mM)	Before stimulus	During stimulus	No. of cells	stimuli	the cell (mM)*
10	0.89 ± 0.31	0.23 ± 0.07	8	100	0.52
1	0.89 ± 0.50	0.25 ± 0.05	9	173	0.022
0.1	0.90 ± 0.47	0.32 ± 0.09	10	164	0.0044
0.01	1.18 ± 0.34	0.92 ± 0.23	3	26	< 0.0005
0	1.04 ± 0.10	1.00 ± 0.49	3	31	0

Measurements were made as described in Figs 1 and 2 legends, with -100 nA pulses of length 0.3-5 s. Each cell was tested at only one concentration of α -MeAsp in the pipette. Values for the CW interval are the mean and s.d. of the mean intervals for each cell, each cell weighted

* Estimated from the transition time, t, for adaptation to a continuous -100 nA pulse, using the formula C = 0.16 t/(360 - t), where C is in mM, t in s (see Fig. 2 of ref. 20). The fractional change in receptor occupancy is $C/(K_d + C)$, where K_d is the apparent dissociation constant of the receptor.

gene products)28. Our results suggest that the cheZ product lies on the excitation pathway. Alternatively, its binding to the flagellar motor is required for rapid initiation of CCW rotation.

The lack of correlation between latency and the directional bias of the motor observed in wild-type cells also seems to be the case for cells containing cheR-cheB or cheZ mutations. The time required for a cell to adapt to a step change in the concentration of α -MeAsp, however, is strongly correlated with the directional bias of the motor (with the mean CCW interval)29. This implies that the signals involved in excitation and adaptation reach the motor by separate pathways or that the latency is due to a step that occurs after these pathways merge.

Although cheZ mutants generally have abnormally high tumbling rates, cheZ-cheC double mutants exist that fail to move up gradients even though their tumbling rates are normal³⁰. Mutants of this sort may represent a new kind of che phenotype. in which the defect lies in the rates at which cells respond to time-varying stimuli. If the latency of a cell is longer than its mean run length, it will choose a new direction at random before it has had a chance to make the appropriate response.

Why is the latency of wild-type cells as long as 0.2 s? There is no reason, a priori, that the response to a large stimulus could not occur within a few milliseconds. However, a cell must time-average its measurement of receptor occupancy if it is to sense small changes in concentration in the presence of statistical fluctuations. For most of the stimuli encountered in nature, averaging times of hundreds of milliseconds are required³¹ There may never have been any selective pressure for the development of more rapid signal-processing machinery.

We thank Steve Block, Jim Hudspeth, Victor Neher and Bob Smyth for valuable suggestions and Sandy Parkinson for bacterial strains. This work was supported by grant AI16478 from the US NIH.

Received 14 December 1981, accepted 15 March 1982

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Low doses of ethanol disrupt sensory responses of brain noradrenergic neurones

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Ethanol is a widely abused drug which has many behavioural and psychological effects¹. In spite of considerable research¹ the brain mechanisms responsible for these effects are unknown. Previously, it has been proposed that noradrenaline (NA)containing locus coeruleus (LC) neurones, which project throughout the brain^{5,6}, mediate the effects of many abused as well as clinically effective psychoactive agents7,8. Recent studies⁹⁻¹¹ have shown that in freely behaving, undrugged animals, NA-containing LC (NA-LC) neurones exhibit marked, short-latency responses to sensory stimuli of many modalities, perhaps serving to bias brain and behavioural activities towards adaptive responses to phasic, unexpected environmental events. We have now examined the effects of ethanol on these sensory responses of NA-LC neurones. In the present study, anaesthetized animals were used to minimize fluctuations in arousal, providing a more stable baseline for assessing pharmacological effects. A class of NA-LC sensory responses which mimic those observed in unanaesthetized animals was studied. In addition, using antidromic stimulation, we investigated the effects of ethanol on the soma excitability, axonal conduction velocity, and strength of recurrent collateral inhibition of these neurones. We now report that low intoxicating doses of ethanol substantially reduce the magnitude and temporal reliability of sensory-evoked responses in NA-LC neurones, perhaps due in part to enhanced feedback inhibition of these cells.

Action potentials were recorded from single NA-LC neurones using glass micropipettes in anaesthetized, male adult albino rats. Rectal temperature was maintained between 36

and 37 °C using a thermistor-controlled heating pad. The locus coeruleus was approached at a 30° caudo-rostral angle to avoid the overlying transverse sinus. The experimental paradigm consisted of recording at least 3 min of spontaneous discharge, followed by activity during a 1 Hz train of 100 antidromic stimuli to either the medial or lateral neocortex, or to the dorsal noradrenergic bundle. Thirty second later activity was recorded

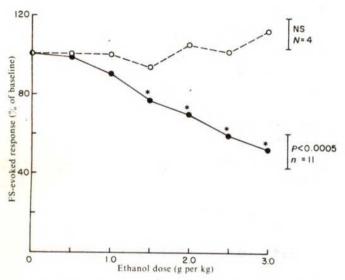


Fig. 1 Effects of ethanol on the magnitudes of FS-evoked response. Response magnitudes are plotted for cumulative doses of ethanol (\bullet) as per cent of baseline (that is, before ethanol treatment) values. Data for similar consecutive trains of FS stimulation without ethanol administration are also shown (\bigcirc). Data from animals anaesthetized with chloral hydrate (n=5) and halothane (n=6) are pooled. NS, no statistically significant effect of stimulation alone on response magnitudes. Bars represent mean \pm s.e.m. and P values are confidence levels resulting from one-way analysis of variance over ethanol doses. *P < 0.005, paired t-tests for each dose compared with baseline.

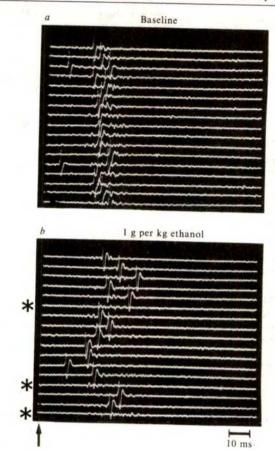


Fig. 2 Effect of ethanol on the latencies of FS-driven spikes. Oscilloscope sweeps triggered consecutively from top to bottom by 1 Hz FS stimuli (arrow) before (a) and 10 min after 1 g per kg ethanol i.p. (b) for the same NA-LC neurone. Note the three sweeps in b that have no spikes (indicated by asterisks) and the increased variability in the latency of FS-driven spikes compared with pre-ethanol activity in a.

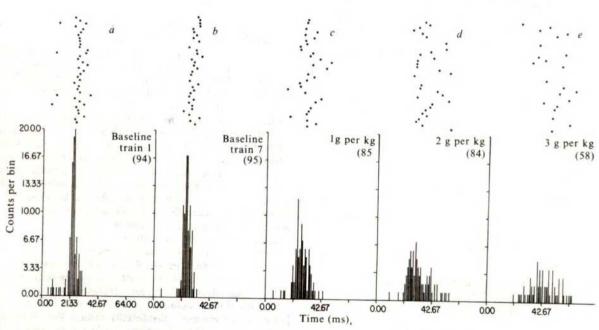


Fig. 3 Post-stimulus time histograms (PSTHs) and raster displays of activity in the same FS-stimulated NA-LC neurone before and after administration of cumulative ethanol doses. a, b, PSTHs for the first and seventh trains of 100 pre-ethanol stimuli and corresponding raster displays for 35 of these stimuli (in consecutive order from top to bottom). c-e, Data following cumulative doses of ethanol, as shown. Numbers in parentheses are the number of FS-driven spikes occurring in each corresponding PSTH. Note that the increased latency variability of FS-driven activity can occur without substantial change in the magnitudes of driven responses (compare c with d). PSTH time bases also apply to corresponding raster displays.

during a similar train at 10 Hz and a subsequent 1 Hz train of 100 electrical stimuli to the contralateral foot (foot-shock, FS). Responses were quantified using computer-generated post-stimulus time histograms (PSTHs)⁹. Response magnitude was defined as the number of spikes within a specified PSTH response interval. After determination of baseline values, 0.5–1.0 g ethanol (12% aqueous solution) per kg was injected intraperitoneally (i.p.), and then the series of tests was repeated. Ethanol injections and test series were made at 10 min intervals, up to a maximum cumulative dose of 3 g per kg. Only one cell per animal was tested with ethanol, and blood ethanol levels were verified at the end of the recording sessions to be appropriate for the injected dose. All recordings were histologically localized to the LC by iontophoresing Pontamine Sky Blue from the tip of the micropipette at the recording site.

Ethanol had no statistically significant effect on the mean spontaneous discharge rate of NA-LC neurones across the entire dose range tested in animals anaesthetized with either chloral hydrate or halothane. Similarly, ethanol had no effect on the ability of 1 Hz antidromic stimulation to elicit soma impulses in these neurones. Pronounced effects of ethanol were seen, however, on the ability of electrical FS stimulation to drive NA-LC neurones orthodromically. As shown in Fig. 1, ethanol decreased in a dose-dependent manner the magnitudes of responses elicited by FS stimuli, an effect observed in each NA-LC neurone tested. Response magnitudes significantly reduced by a lower dose of ethanol (1 g per kg) in chloral hydrate- than in halothane-anaesthetized (1.5 g per kg) animals. The decrease in number of FS-driven spikes was the result of neither repeated trains of FS stimulation nor prolonged anaesthesia (see Fig. 1).

In addition to reducing response magnitudes, ethanol increased substantially the variability of trial-to-trial response latencies exhibited by individual cells (Figs 2, 3). This may be a more profound effect of ethanol on NA-LC neurones than the decrease in response magnitudes, because the standard deviation of response latencies increased to a much greater extent from baseline values (325% after 3 g per kg; see Fig. 4) than the response magnitudes decreased (55% below baseline after 3 g per kg ethanol). In addition, the increase in the standard deviation of latencies was significantly different from baseline at a lower dose of ethanol than was the decrease in response magnitude (compare Figs 1, 4). Thus, the temporal reliability of responses was reduced in a dose-dependent manner for NA-LC neurones after the administration of ethanol, but more markedly and at lower doses than the parallel decreases in response magnitudes. Furthermore, these results for single cells indicate that the temporal coherence (that is, synchronous response activity) characteristic of NA-LC neurones may be markedly reduced by these low doses of ethanol.

A mechanism by which ethanol may exert these effects on NA-LC neurones is suggested by its effects on antidromically elicited activity. Although ethanol had no effect on the number of soma spikes elicited by 1 Hz antidromic stimulation, antidromic soma activation by 10 Hz stimulation and the spontaneous activity which follows antidromic invasion were both reduced by ethanol in a similar dose-dependent manner, to \sim 60 and 70% of baseline, respectively, after 3g per kg ethanol (P < 0.01 and < 0.05, respectively; one-way analysis of variance, n = 5 for all measurements). As both these effects are indices of potency of the recurrent collateral inhibition previously proposed for NA-LC neurones¹², the results indicate that ethanol may increase such recurrent inhibition following activity within the NA-LC. This increased inhibition may partially explain the reduced sensory responsivity we found for these neurones.

In addition we found that ethanol caused a significant dosedependent increase of >8% in the latency of antidromic initial segment spikes in NA-LC neurones during 1 Hz activation. This increased lability in conduction velocity along NA-LC axons seems to enhance the normal variability of conduction

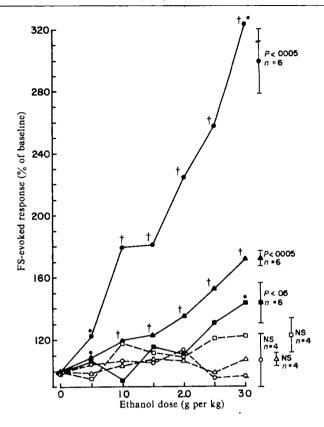


Fig. 4 Effect of cumulative ethanol doses on various measures of FS-driven activity in single NA-LC neurones FS-response latency values. \bullet , s.d. for ethanol treatment; \bigcirc , s.d. for control data (obtained for similar trains of FS stimulation in the absence of ethanol); \blacktriangle , mean for ethanol; \triangle , mean for control; \blacksquare , onset for ethanol; \square , onset for control. Data are expressed as per cent of baseline values. Note that the most substantial effect of ethanol is to increase the variability (expressed here as s.d.) of latency to FS-driven activity, and that there is only minimal effect on onset latencies. Bars represent mean \pm s.e.m. and P values are confidence levels resulting from one-way analysis of variance for dose of ethanol, or for number of stimulus trains in the case of control data. NS, not statistically significant. *P < 0.05; †P < 0.005, paired t-tests for dose or stimulus train compared with baseline data.

velocity during low-frequency impulse activity previously reported for these neurones¹³.

Our results showing decreased sensory responsiveness of NA-LC neurones after low doses of ethanol suggest a new interpretation of previous reports of the effects of ethanol on NA-LC discharge. In one study in paralysed rats14, most LC neurones exhibited decreased activity after a 2 g per kg dose. However, the cells were found to discharge spontaneously at ~17 Hz, much more rapidly than is typical of NA-LC neurones in active animals⁹⁻¹¹ or in anaesthetized rats^{12,13,15-19}. If this elevated activity is due to increased tonic orthodromic input to NA-LC neurones (perhaps as a result of stress induced in paralysed, artificially respired, unanaesthetized animals), then the decreased activity following ethanol administration would be consistent with our results of reduced sensory responsivity in these cells after ethanol. This interpretation agrees with previous results²⁰ in anaesthetized rats, in which more characteristic rates of spontaneous activity for NA-LC neurones were unaffected by ethanol.

In the present studies, quantitatively similar effects of ethanol on NA-LC discharge were observed using either chloral hydrate or halothane anaesthesia; similar results were also observed for three animals anaesthetized with urethane. The fact that these results are reproducible with different anaesthetics indicates that these ethanol effects are not due to an interaction of ethanol

with the anaesthetic and that similar effects may be observed in waking preparations. The fact that doses of ethanol (for example, 3 g per kg) that are greater than those yielding significant reductions in sensory responsiveness in our studies had no effect on the ability of 1 Hz antidromic spikes to invade the soma-dendritic membrane indicate that our results are not due to a general membrane effect on these neurones, such as decreased excitability of NA-LC somata. In addition, previous studies21 indicate that effects of ethanol on peripheral nerve conduction are unlikely to account for our results. The lack of effect on spontaneous discharge rate provides additional evidence that ethanol at these doses is not acting in a nonspecific manner, and also indicates that the increase in intra-coerulear collateral inhibition we propose is not sufficiently intense to alter discharge during spontaneous, unsynchronized activity. Rather, this effect appears to become manifest only when collaterals are synchronously activated, such as during antidromic or sensory stimulation when a large percentage of NA-LC neurones would be engaged simultaneously.

In conclusion, our results demonstrate that low intoxicating doses (0.5-1.0 g per kg) of ethanol produce a marked decrease in the magnitude of sensory-evoked activity in NA-LC neurones and substantially increased variability in the latency of these responses, while also increasing the lability of impulse conduction along their axonal projections. Our results further lead us to hypothesize that ethanol increases the potency of recurrent collateral inhibition within the NA-LC, an effect perhaps responsible in part for the reduced sensory responsiveness of these neurones. We cannot exclude, however, the possibility that ethanol may also decrease the efficacy of excitatory afferents to the locus coeruleus.

Our results suggest a possible mechanism for many of the actions of ethanol on the brain. The diverse efferent system of projections originating from NA-LC neurones^{5,6} and the pronounced effects of NA on locus coeruleus target cells²²⁻²⁵ indicate that a change in the discharge characteristics of NA-LC neurones would have widespread consequences on the central nervous system and on behaviour. Previous studies on the normal discharge properties of NA-LC neurones in behaving animals9-11 have suggested that this system functions to bias the mode of brain information processing and the orientation of behaviour to favour either phasically adaptive responses to unexpected external stimuli or more tonic, vegetative behavioural programmes^{26,27}. Significant disorganization of such a function, an implied action of ethanol in the present studies, may underly the marked disruption by ethanol of adaptive behavioural activity.

Received 12 November 1981; accepted 17 February 1982

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Conduction block in the peripheral nervous system in experimental allergic encephalomyelitis

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Experimental allergic encephalomyelitis (EAE) has been widely studied as a model of multiple sclerosis, a central nervous system (CNS) disease of unknown actiology. The clinical features of both EAE and multiple sclerosis provide the only guide to the progress and severity of these diseases, and are used to assess the response to treatment. In such comparisons the clinical features of EAE are assumed to be due to lesions in the CNS, but in this disease there is also histological evidence of damage to the peripheral nervous system1-8. However, the functional consequences of such peripheral lesions have been entirely ignored. To examine this we have studied nerve conduction in rabbits with EAE. We report here that most of the large diameter afferent fibres are blocked in the region of the dorsal root ganglion and at the dorsal root entry zone, thus accounting for the loss of tendon jerks and also, through the severe loss of proprioceptive information, the ataxia of these animals. We conclude that whenever clinical comparisons are made between EAE and multiple sclerosis, the pathophysiology associated with the histological damage of the peripheral nervous system must be taken into account.

EAE is a disease induced by inoculating with CNS-derived antigens and adjuvants, and is characterized clinically by limb ataxia and weakness9 and histologically by CNS lesions consisting of meningeal infiltration with mononuclear cells, perivascular cuffing and para-adventitial infiltration with mononuclear cells and perivascular demyelination^{9,10}. This disease has been studied extensively, especially recently in its chronic relapsing form11, because it is widely accepted as an animal model of multiple sclerosis¹². However, it has been assumed that the clinical features of EAE are due to CNS lesions even though histological damage of the peripheral nervous system occurs in many species such as the rabbit^{3,4}, mouse⁵, guinea pig^{1,5} and monkey² and when antigens derived solely from the CNS are used3. It also occurs in rabbits with chronic EAE6, and in guinea pigs⁷ and rats ⁸ with chronic relapsing EAE. Thus, it is possible that the clinical features of EAE are due to lesions of the peripheral rather than the central nervous system. This question needs resolving because the improvement or suppression of the clinical features of EAE by agents such as myelin basic protein¹ and immunosuppressants 14 provides the basis for their use in the treatment of multiple sclerosis.

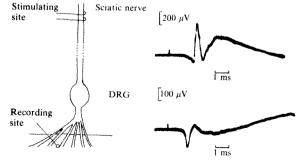


Fig. 1 Recording in the volume conductor over the S1 dorsal root entry zone in response to sciatic nerve stimulation. In each trace, positivity at the active electrode gives a downward deflection. Upper trace, normal rabbit; lower trace, rabbit with EAE. Note the difference in gain. DRG, dorsal root ganglion.

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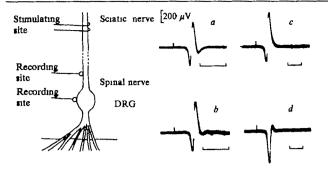


Fig. 2 Recordings in the volume conductor over the spinal nerve and the DRG in response to sciatic nerve stimulation. a, Recording over S1 spinal nerve in normal rabbit. b, Recording over normal S1 DRG. c, Recording over S1 spinal nerve of rabbit with EAE. d, Recording over S1 DRG from the same rabbit with EAE. Time bars, 2 ms.

We have therefore made a clinical, physiological and histological study of EAE in New Zealand white rabbits inoculated intradermally with homogenized rabbit spinal cord and complete Freund's adjuvant. The clinical onset occurred 15-23 days after inoculation and was typical of that previously described', with weight loss, ataxia and weakness of the limbs occurring over 3-4 days. We also examined the tendon jerks and found that these usually disappeared about 2 days after the clinical onset, indicating a lesion interrupting the monosynaptic reflex arc. All the clinical findings could thus be accounted for by lesions in the peripheral nervous system. Histological examination revealed typical, widespread CNS lesions as well as lesions in the dorsal root ganglia and dorsal and ventral roots.

In terminal experiments, 2-6 days after clinical onset, animals were anaesthetized intravenously with 25% urethane, a lumbosacral laminectomy was performed and the left sciatic nerve was exposed in the posterior thigh. The left medial gastrocnemius compound muscle action potential elicited by stimulation of the sciatic nerve in the mid-thigh was normal in all of 12 animals studied.

Conduction into the roots and spinal cord was studied in 10 affected animals. Figure 1 shows typical responses recorded in the volume conductor over the left S1 dorsal root entry zone in response to sciatic nerve stimulation. The upper trace (from a normal control animal) shows a triphasic (positive–negative–positive) wave representing the afferent volley, followed by a longer latency, slow negative wave, the N wave. The initial positive wave is due to the passive outward currents driven by the last activated nodes of Ranvier; the negative phase of the afferent volley is due to the active inward currents occurring during the rising phase of the action potentials and ranges from 300 to $800 \,\mu\text{V}$ in amplitude. The peak-to-peak amplitude of the triphasic wave ranges from $400 \, \text{to} \, 1,000 \, \mu\text{V}$. The N wave is due to the synaptic currents in the second order dorsal horn neurones excited mainly by low threshold cutaneous afferents.

The lower trace in Fig. 1 (from an affected animal) shows three characteristic abnormalities. (1) A very reduced peak-to-peak amplitude (usually <200 μV) indicating that only a small proportion of the large diameter afferent fibres are conducting past the dorsal root ganglion to this point. (2) A very reduced (usually <50 μV) negative wave and a relative increase in the positive wave of the afferent volley, indicating that most of the large diameter fibres still conducting past the ganglion are blocking in the vicinity of the dorsal root entry zone. (3) A delayed peak of the N wave possibly due to conduction persisting in small fibres.

To determine the site of conduction block, volume conductor recordings were made over the spinal nerve and dorsal root ganglion (Fig. 2); traces a and b are are the responses over the normal S1 spinal nerve and S1 dorsal root ganglion, respectively. They are similar and consist of an initial positive wave followed by a larger negative wave. Figure 2c shows a recording

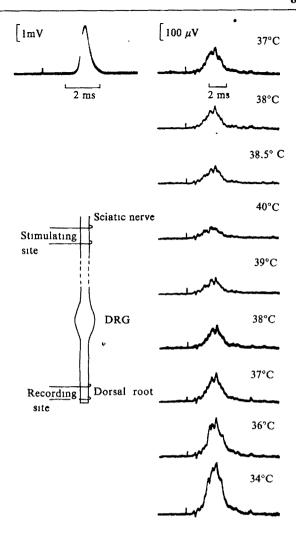


Fig. 3 Monophasic recording from the distal cut end of S1 dorsal root in response to sciatic nerve stimulation. Upper left trace, normal rabbit. Traces on the right are recordings from a rabbit with EAE at various laminectomy pool temperatures, as indicated on the right. Note the difference in gain and time base.

over the S1 spinal nerve of a rabbit with EAE and is normal. The recording in Fig. 2d was obtained over the S1 dorsal root ganglion of the same affected animal and shows a large positive wave followed by a small negative wave, indicating that most of the fibres are blocking at this point.

To investigate the nature of the conduction block, the effects of temperature were studied. Recently, it has been shown 15,16 that in demyelinated single fibres, increasing the temperature within the physiological range produces reversible conduction block, and that in some fibres conduction block can be overcome by reducing the temperature by as little as 0.5 °C. The method used to study the effects of temperature is shown diagrammatically in Fig. 3. The distal cut end of the S1 dorsal root was placed on a pair of electrodes and a monophasic recording made of the compound action potential in response to sciatic nerve stimulation. The upper left trace is from a normal anaesthetized rabbit. The right-hand traces were made at various temperatures of the laminectomy oil pool of a rabbit on the fifth day of clinical disease. On the previous day we increased the body temperature of this animal by 1.5 °C—this caused a reversible increase in ataxia. The response at 37 °C was greatly reduced in amplitude (note the difference in gain) with an area less than 30% of the normal compound action potential, suggesting that most of the large diameter fibres were blocking in

the region of the dorsal root ganglion. The area of the response was progressively reduced as the temperature was increased to 40 °C, which indicates that block was occurring in an increasing number of fibres. When the temperature was reduced to 37 °C, the response regained its original area. On further cooling to 34 °C the area increased by 100%, showing that conduction was restored in a significant number of fibres. These findings strongly suggest that the conduction block is due to demyelination

From our studies we conclude that the loss of tendon jerks and the ataxia of rabbits with EAE can be accounted for by conduction block in most of the large diameter afferent fibres in the region of the dorsal root ganglion and dorsal root entry zone. Such conduction block would mask the clinical expression of any central lesions which alone could produce ataxia. Clearly, these functional considerations must be taken into account when making a clinical comparison between EAE and multiple sclerosis

We thank Drs L. Cuzner and C. Bolton for advice concerning the inoculation procedure. Support from the National Fund for Research into Crippling Diseases and the Brain Research Trust is gratefully acknowledged.

Received 18 January; accepted 9 March 1982

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Differences in the kinetics of rod and cone synaptic transmission

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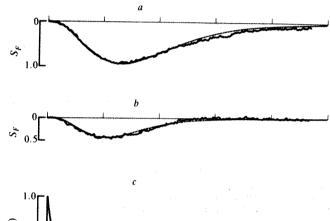
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Photoreceptors of the vertebrate retina hyperpolarize in response to light. The hyperpolarization elicited by a brief flash is approximately ten times slower in rods than in cones of the same retina1. We have examined the amplification and temporal properties of synaptic transfer of rod and cone signals to a common postsynaptic element, the horizontal cell. We find that the kinetics of signal transfer at these chemical synapses parallels the speed of the light-evoked signals themselves.

Synaptic transfer was studied by recording simultaneously the responses of pre- and postsynaptic cells to dim flashes of light. Eyecups from the snapping turtle (Chelydra serpentina) were prepared as described earlier2. Two intracellular microelectrodes, with tips -100 µm apart in the plane of the retina, were independently lowered into the retina. Intracellular recordings were made from photoreceptors and from axon

terminals of luminosity horizontal cells (HIAT). HIATs receive synaptic input from both rods and red-sensitive cones^{3,4}. Cells were classified on the basis of their receptive field properties and response waveforms, criteria which have been justified with intracellular dye injections^{2,4,5}. Changes in membrane potential were recorded in response to brief (13 ms) flashes of light at a wavelength of 520 or 650 nm. The light intensities used were shown experimentally to be in the linear range¹. Large circular spots of light (1.5-4.3 mm diameter) were utilized in order to eliminate temporal filtering of signals caused by coupling between photoreceptors and between horizontal cells^{6,7} rod-horizontal cell pairs and five cone-horizontal cell pairs were used in this analysis.

Stimulation of cones with bright lights elicited membrane hyperpolarizations in the horizontal cells of up to 40 mV, while rods elicited horizontal cell hyperpolarizations of only a few millivolts. The cone- and rod-mediated inputs to horizontal cells were adequately separated by using stimuli of different wavelengths. The horizontal cell response to dim 520-nm test flashes was mediated solely by rods, as no measurable response in a cone was ever recorded to test flashes of the intensities used to stimulate rod-horizontal cell pairs. Although 650 nm light excited both rods and cones, the faster cone contribution to the horizontal cell response could be clearly separated from that of the rod4. To confirm that the initial part of the 650-nm



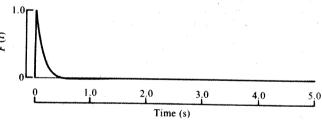


Fig. 1 The rod-horizontal cell synapse. Results from 63 test flashes, 520 nm, presented at t = 0. Two test flash intensities were chosen in order to test for intensity/response linearity. The intensity of the first 30 flashes was 1.0 photon per μm^2 per flash; the intensity of the second 33 was 0.5 photons per μ m² per flash. Stimulus diameter 3.8 mm; S_F , in units of mV per photon per µm² per flash, was obtained by dividing the voltage responses by the flash intensity. a, Ensemble average of light responses of rod. Dashed curve fitted to average is the equation $S_F(t) = A(te/T)^n \exp(-tn/T)$, where A is the sensitivity at the peak of the light response in mV per photon per μ m² per flash, T is the time to the peak of the response in seconds and n is an integer chosen to give the best fit by eye of the experimental data to the curve⁹. In some cases, a non-integer value of n gave a better fit to the rising phase of the light response, but the deconvolution procedure described below requires an integer value. However, the selection of an integer above or below this value has only a small effect on the duration of the calculated impulse response. A = 0.90, T = 1.34 and n = 4. b, Ensemble average of light responses of horizontal cell. Responses recorded simultaneously with rod responses for a above. Dashed curve fitted to average is the same equation as for a, with A = 0.45, T = 1.06 and n = 5. c, Normalized impulse response of rod-horizontal cell synapse. The curve is calculated by deconvolving the equations fitted to the horizontal cell and photoreceptor light responses. The solution to that deconvolution is

$$F(t) = C \exp(-tn_2/T_2) \sum_{k=0}^{n_1+1} t^{(n_2-k)} \frac{(n_1/T_1 - n_2/T_2)^{-k}}{(n_2-k)! \, k! \, (n_1+1-k)!}$$

where C is a constant chosen to normalize F(t) to one at its peak, A, nand T are the constants described above, with subscripts 1 and 2 referring to the values for the photoreceptor and horizontal cell respectively.

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response was not contaminated by rod inputs, the 650-nm test flash was presented on dim red or green backgrounds whose intensities were adjusted for equal cone absorption. In these conditions, cone responses and the early portion of the horizontal cell responses were independent of the wavelength of the background light.

Averaged responses to dim test flashes are shown in Fig. 1 for a rod-horizontal cell pair, and in Fig. 2 for a cone-horizontal cell pair. In all pairs recorded, the response of the photoreceptor peaked after the response of the horizontal cell. On average, the peak of the cone response followed the peak of the conedriven response of the horizontal cell by 8 ms (range 2-18); the peak of the rod response followed that of the rod-driven response of the horizontal cell by 425 ms (range 280-600). The gain of synaptic transfer (the ratio of peak horizontal cell amplitude to peak photoreceptor amplitude) was 5.0 for the horizontal cell-cone pairs (range 2-10) and 0.46 for the horizontal cell-rod pairs (range 0.4-1.2). These values are comparable with those obtained by sequential recording of cones and horizontal cells8, but smaller than the rod-horizontal cell gains previously described4.

To determine how the waveform of the light response of a photoreceptor is transformed during its transfer to the horizontal cell, we calculated the impulse response of the synapse, F(t). This is the calculated form of the voltage response in the horizontal cell for a brief change in voltage in the photoreceptor, and it reflects the rate-limiting steps in synaptic transfer.

Figures 1c and 2c are plots of the normalized impulse response. The method for obtaining this function is described in Fig. 1 legend. The impulse responses of rod and cone synapses are similar in shape and differ only in time scale. The impulse response rises immediately at zero time and slowly relaxes back to baseline with a small undershoot. This waveform is characteristic of a leaky high-pass filter in series with a low-pass filter.

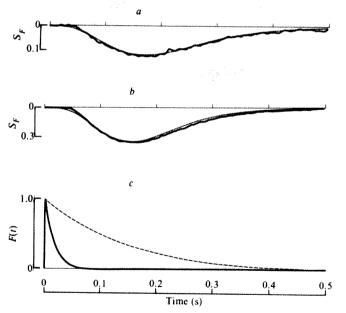


Fig. 2 The cone-horizontal cell synapse. Results from 53 test flashes, 650 nm, presented at t = 0. The intensity of the first 23 flashes was 4.0 photons per µm² per flash, and of the second 30 flashes was 8.9 photons per flash. Stimulus diameter 3 mm, approximately twice the diameter of the receptive field of the horizontal cell. S_F is in units of mV per photon per μ m² per flash. Note that the time scale has been expanded 10 times relative to Fig. 1. a, Ensemble average of light responses of cone. Dashed curve fitted to average is the equation in Fig. 1a legend with A = 0.115, T = 0.174 and n = 5. b, Ensemble average of light response of horizontal cell. Responses were recorded simultaneously with the cone response for a above. Dashed curve fitted to average is the equation in Fig. 1a legend, with A = 0.345, T = 0.156 and n = 6. c, Normalized impulse response of cone-horizontal cell synapse (the smooth curve). The curve is calculated from the equation in Fig. 1c legend using the values given in 2a and 2b as the constants of the equation. For comparison, the impulse response in Fig. 1 of the rod synapse is re-plotted on the faster time scale (---).

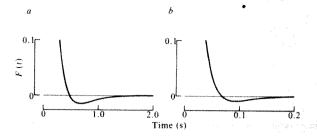


Fig. 3 Undershoot of the impulse response. Portions of the impulse response in Figs 1, 2 are redrawn at higher magnification to illustrate the small undershoot. a Is the impulse of the rod-horizontal cell synapse of Fig. 1, and b is that of the cone-horizontal cell synapse of Fig. 2.

High-pass filtering (or differentiation) is required to account for the faster time to peak of the horizontal cell response relative to the photoreceptor response; the high-pass filtering is also responsible for the negative component of the impulse response which can be seen to dip just below the baseline on the magnified scale in Fig. 3. Low-pass filtering is required to fit the rising phase of the horizontal cell response. At early times the light response of the photoreceptor rises as approximately t^n , while the horizontal cell response rises as t^{n+1} . (Each low-pass stage through which the photoreceptor signal is filtered adds an additional power of t to the rising portion of the horizontal cell response⁹.)

The nature of synaptic filtering is unclear. The high-pass filter stage may reflect time- and voltage-dependent behaviour of the postsynaptic channels 10, postsynaptic 'desensitization' presynaptic depletion of transmitter¹² due to prolonged release. The low-pass filtering stage may represent the finite time that postsynaptic channels are opened by the transmitter released from photoreceptors¹³. In cone-horizontal cell pairs, the lowpass filtering might also simply reflect the filtering of the membrane voltage of the horizontal cell by its membrane time constant¹⁴. The kinetics of transmitter release, degradation and uptake at synapses in other physiological systems are fast relative to channel opening and membrane time constants 13,15, but these processes may be slower at the photoreceptor synapse.

The duration of the synaptic impulse is much greater for the synapse between rods and horizontal cells than between cones and horizontal cells: the impulse response falls to 1/e of its peak value in, on average, 131 ms for the rod synapse (range 100-160) and in 16 ms for the cone synapse (range 12-20). In comparison, in an analysis of the fluctuation of membrane potentials of bipolar cells in the same retina16, the impulse for the cone-bipolar cell synapse was estimated to last ~14 ms in the hyperpolarizing bipolar and ~50 ms in the depolarizing bipolar. A small degree of high-pass filtering was also observed in the bipolar synapses.

The time scale of the synaptic impulse from rod to horizontal cell is approximately 10 times slower than in the conehorizontal cell pair. This difference parallels a difference in the speed of light responses of rods and cones. It was previously suggested that the match between the temporal properties of the light response of photoreceptors and the kinetics of synaptic transmission to ganglion cells might improve the signal-to-noise ratio of transmission in the retina¹⁷. The present experiments demonstrate that this kind of optimization is already apparent at the first synapses of the retina.

We thank Drs J. F. Ashmore, D. A. Baylor, B. J. Nunn and D. G. Green for comments on the manuscript, and Ms J. Ansiello for typing the manuscript. This research was supported by NIH grants NS-07067 and EY-01869.

Received 19 November 1981; accepted 16 March 1982

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Globin structural mutant $\alpha^{125 \text{Leu} \rightarrow \text{Pro}}$ is a novel cause of α -thalassaemia

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The duplicated human α -globin structural loci lie on chromosome 16 and are arranged in the order 5' $\alpha 2-\alpha 1$ 3'. Although the most common molecular mechanism for α -thalassaemia is deletion of segments of DNA that contain one or both of these α -globin structural loci¹, two molecular lesions that are not due to gross gene deletion have been defined. In one, a point mutation in the termination codon of the $\alpha 2$ gene results in the production of an elongated α -globin chain^{2,3}. As only a small amount of the mutant globin chain is produced, α thalassaemia results. The second lesion, which is caused by a 5-base pair (bp) deletion in the first intervening sequence of the α 2 gene, results in abnormal mRNA processing and α globin chain deficiency4. We now describe a novel mechanism for α -thalassaemia not involving deletion. A single nucleotide mutation in the coding region of the $\alpha 2$ gene results in the substitution of proline (Pro) for leucine (Leu) in a region of the H helix of the α -globin chain, which is critical for $\alpha 1-\beta 1$ contact. This probably impedes $\alpha 1-\beta 1$ dimer formation, the initial step of haemoglobin tetramer assembly, and produces an α -thalassaemia phenotype.

The clinical, biochemical and genetic data on a particular patient with haemoglobin-H (Hb-H) disease and his family have been reported in detail elsewhere5. Usually only one of the four α -globin loci remains in Hb-H disease (genotype --/- α); the propositus and his sister, however, have Hb-H disease with both α-globin loci deleted on one chromosome and intact on the other (genotype $--/\alpha\alpha$). The family members who have microcytosis characteristic of α -thalassaemia have two different genotypes: the usual two-gene deletion $(--/\alpha\alpha)$ and an unusual genotype in which all four α -globin genes are intact $(\alpha \alpha / \alpha \alpha)$. To explain the presence of α -thalassaemia trait in members of the latter genotype, we postulated that one of the chromosomes with two intact α -globin genes was defective in α -globin chain production $[\alpha \alpha/(\alpha \alpha)^{T}]$, and that the combination of this nondeletion α -thalassaemia chromosome with the usual two-gene deletion form produced the Hb-H phenotype seen in the propositus and his sister $[--/(\alpha\alpha)^{T}]$.

Previous Southern analysis of the propositus' DNA revealed no abnormalities in the α -globin gene map⁶. mRNA analysis showed an α 1- to α 2-globin mRNA ratio of 2.5:1, which is identical to that found in normal reticulocytes, and indicates that both α -globin genes are active in transcription⁷. To define the molecular lesion producing non-deletion α -thalassaemia, we cloned the segment of his DNA that contained the two α -globin genes. This segment must be derived from the chromo-

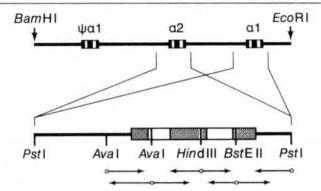


Fig. 1 Diagram showing the DNA segment cloned from the patient with non-deletion Hb-H disease, and the sequencing strategy of the α loci. Leukocyte DNA from the propositus was digested with BamHI and the 14.5-kb fragment isolated by discontinuous electrophoresis on an agarose gel14. The BamHI end was made flush by adding the tetranucleotide GATC with Klenow fragments of DNA polymerase I, and was ligated to an EcoRI . The DNA was then digested with EcoRI and the resultant 13-kb fragment containing the 5' $\psi\alpha$ - α 2 and α 1 3' loci was ligated to Charon 4A arms at the *EcoRI* site. The phage was packaged and used to infect *Escherichia coli* DP50 Sup F¹⁶. Phages containing α -globin inserts were identified by the method of Benton and Davis 17 with 32P-labelled α-globin cDNA, and those with a positive signal were screened twice more. One such isolate, AGL6, was grown in a 11 culture. The phage particle was isolated and banded on a caesium chloride gradient (1.5 g ml⁻¹) in a Ti50 rotor at 30,000 r.p.m. for 20 h. The DNA was then digested with EcoRI and BglII, and two fragments of 9.2 and 3.6 kb, which contained the $\psi\alpha$ - α 2- and α 1-globin loci respectively, were subcloned into the EcoRI-BamHI sites of pBR322. The restriction sites used for sequencing are shown in the lower diagram. Sequencing was performed according to the Maxam and Gilbert method18, and carried out side by side with the corresponding fragments from the normal α -globin loci cloned by Lauer et al.

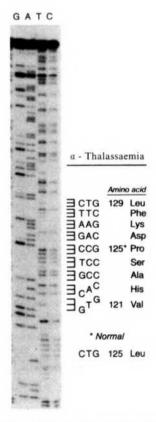


Fig. 2 Sequencing gel of the patient's DNA showing the region of the mutation. The DNA fragment was 5'-labelled at the BstEII site with 32P by polynucleotide kinase, and sequenced by the method of Maxam and Gilbert.

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some affected by non-deletion α -thalassaemia, as both α -globin loci are deleted from the other chromosome. We cloned in Charon 4A a 13-kilobase (kb) fragment extending from the BamHI site to the EcoRI site and containing the $\psi \alpha$ -, α 2- and α 1-globin genes. The α 1- and α 2-globin loci were then separately subcloned into pBR322. Both the α 2- and the α 1-globin genes were sequenced side by side with the corresponding α -globin fragments from a previously isolated normal DNA. (See Fig. 1 for details.)

We completely sequenced the $\alpha 1$ and $\alpha 2$ loci, from the AvaI site located 100 nucleotides 5' to the cap site to the PsfI site 90 nucleotides 3' to the poly(A) addition site. The α 1-globin gene sequences from the propositus' DNA were identical with normal α1 sequences. We detected one difference, however, in the coding region of the $\alpha 2$ gene, at the position corresponding to amino acid 125, where the normal leucine codon (CTG) was changed to proline (CCG) (Fig. 2). We were able to confirm this mutation by restriction analysis because it abolished an EcoRI recognition site (CCTGG→CCCGG) and created a new recognition site for the enzymes HpaII and MspI (CCGG). The normal 337-bp MspI fragment that extends from the second intervening sequence to the 3'-noncoding region of the a2globin gene was cleaved into two fragments of 216 and 121 bp in the propositus' DNA. The mutation could potentially be detected in this patient's genomic DNA by methods similar to those used to detect the DdeI polymorphism in sickle cell anaemia8.

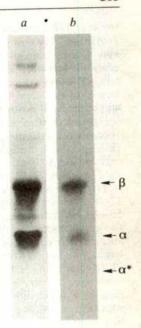
Leu→Pro mutations usually exert a profound effect on the stability of the haemoglobin tetramer. All the known Leu → Pro changes occurring in the α - and β -globin chains produce unstable haemoglobins and cause haemolytic anaemia9. For example, Hb Bibba, an α mutant also located in the H helix $(\alpha^{136\text{Leu} \to \text{Pro}})$, produces an unstable haemoglobin syndrome¹⁰. The unusual feature of the mutation in the family we studied is that the Leu \rightarrow Pro mutation produces α -thalassaemia rather than an unstable haemoglobin. Carriers of this lesion (genotype $\alpha^{125 \text{ Pro}} \alpha / \alpha \alpha$) are phenotypically indistinguishable from carriers of α-thalassaemia trait. The combination of this gene and a deletion α -globin gene $(--/\alpha^{125 \text{ Pro}}\alpha)$ produces the clinical features of Hb-H disease.

A search for the abnormal α-globin chain using electrophoresis on starch gel, Triton-urea gel, and isoelectric focusing was unsuccessful. However, after incubating the patient's reticulocytes with 3H-leucine and separating the haemolysate on a Triton-urea gel, we observed an abnormal radioactive α -globin band that turned over rapidly (Fig. 3). This unstable α -globin could produce Hb-H disease by the following mechanism. Three amino acids ($\alpha^{122\, {
m His}}$, $\alpha^{123\, {
m Ala}}$ and $\alpha^{127\, {
m Lys}}$) in close proximity to $\alpha^{125 \text{ Leu}}$ participate in $\alpha 1-\beta 1$ contact¹¹. The Leu→Pro substitution probably disrupts the H helix and in turn interferes with these contact points. The formation of $\alpha 1 - \beta 1$ dimers may thus be impeded and the ability of the β -chains to form Hb-H (β_4) may exceed that of the abnormal α -chain and the normal β -chain to form $\alpha 1$ - $\beta 1$ dimers. The uncombined α -chains would then be destroyed by proteolysis and an α-thalassaemia-like syndrome would ensue. Such a mechanism is consistent with the normal $\alpha 1$ to $\alpha 2$ ratio found in the propositus7 because the abnormal mechanism operates after translation. In contrast, in all cases of α - and β -thalassaemia produced by abnormal mRNA processing and by early or late termination, mRNA from the affected locus is either decreased or unstable in vivo 4.7,12,13.

In conclusion, we have described a human globin mutation, α 125Leu - Pro, that produces the α-thalassaemia phenotype through a novel post-translational mechanism. As it was the propositus' mother who carried the α -globin mutant, we have named this variant Quong Sze, after the province in China where she was born.

We thank Jennifer Gampell for editorial comments. This study was supported by grants from the NIH and the March of Dimes/Birth Defects Foundation. Y.W.K. is an investigator of the Howard Hughes Medical Institute.

Fig. 3 Electrophoresis of globin on a Triton-urea gel. The reticulocytes from the propositus with Hb-H disease were concentrated by Percoll-Reno M60 gradient²⁰ and incubated with ³H-leucine for 20 min as previously described21. Cells were lysed and 1 µg of haemoglobin was applied to a Triton-urea gel and elec-trophoresed as described earlier22. The gel was stained with Coomassie blue, dried and autoradiographed for 4 weeks. Lane a, Coomassie blue staining of the gel; b, autoradiogram of the gel. The faint radioactive band (α^*) that migrates faster than the normal α -globin band in b has been characterized as the abnormal aglobin by hybrid arrest of translation by α-globin cDNA in a cell-free system (S.A.L. and Y.W.K., unpublished) and work is in progress to assess its synthesis and turnover rates.



Received 5 October 1981; accepted 8 March 1982.

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Germ-line MuLV reintegrations in AKR/J mice

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AKR mice are viraemic from birth1 and contract leukaemia at high incidence at 6-9 months of age2. They express, in all tissues studied, the ecotropic (mouse infectious) retrovirus AKV¹. Rowe^{3,4} showed, by crossing AKR mice with the low-viraemia, non-leukaemic NIH/Swiss mice, that viraemia and the leukaemic phenotype are linked and carried at two independent loci (Akv-1 and Akv-2). These two loci were shown to represent integrated ecotropic proviruses5.6. As part of a study of the structure of proviruses which arise in the leukaemic thymus of AKR mice, we identified ecotropic-specific hybridization probes from different regions of the AKV genome7. Initial Southern hybridization⁸ analyses showed that individual leukaemic AKR/Jackson mice, which have been sibling inbred for over 140 generations, had differing numbers of ecotropic proviruses⁹. We now report analyses showing that AKR/J mice had three common proviruses; however, two additional ecotropic loci were scattered throughout the Jackson Laboratory AKR population. These loci probably arose through reinfection of the germ line and represent a currently occurring amplification of ecotropic proviruses in these inbred mice.

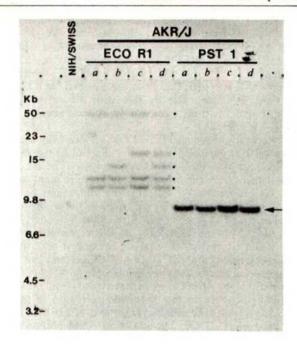
Figure 1 shows a set of four different 'homozygous' hybridization patterns from individual AKR/J mice. The ecotropic virus-specific hybridization probe corresponds to the gp70 region of AKV (Fig. 1). As the AKV genome contains no EcoRI sites⁶, a unique EcoRI fragment, larger than 8.8 kilobases, arises from each integrated AKV provirus. AKR/J mice all carry 50-, 12- and 11-kb ecotropic EcoRI fragments (lanes a-d). The 50-kb EcoRI fragment is the Akv-1 allele6, while the other two are characteristic of the AKR/J substrain6 and have probably been fixed within these AKR mice since the breeding began. In addition to these three loci we observe AKR/J mice which carry a 14- or 17-kb EcoRI fragment (Fig. 1, lanes b, c), some animals carry both proviruses (lane d). We also observe patterns in which either the 14- or 17-kb fragment is heterozygous (appears at one-half intensity, see Fig. 2 as an example). New ecotropic EcoRI fragments have also recently been observed in AKR/J mice by Yoshimura and Breda10.

All five proviral EcoRI restriction fragments seem to contain a full-length AKV or closely related genome. They are larger than 8.8 kb and each hybridizes to the three different ecotropic probes. (Figure 1 shows the three probes on a restriction map of AKV¹¹.) In addition, PstI digestion of each of the four different samples in Fig. 1 (EcoRI lanes a-d) produces a single full-length internal PstI fragment (Fig. 1, PstI lanes a-d), consistent with the restriction map of AKV.

Figure 2 shows that the 14-kb EcoRI ecotropic restriction fragment is inherited in the germ line. A female AKR/Jackson mouse homozygous at all five ecotropic proviral loci was mated with a male AKR/Jackson mouse which lacked the 14-kb proviral EcoRI fragment. EcoRI digestion and Southern blot analysis of the F₁ embryos revealed that the 14-kb fragment was present but at one-half the intensity of the female parent. An analogous experiment showed that the 17-kb fragment is also carried in the germ line (data not shown).

To examine the cellular sequences on each side of the provirus, we used a combination of enzymes, one of which cuts inside the provirus, and a specific probe that lies on a known side of the cut. Figure 3A shows, using the pol probe and a combination of XbaI and EcoRI, that the five 5' sequences are all different. Although the three fixed proviruses generate the same 13-kb XbaI fragment, this is fortuitous, because double digestion with XbaI and EcoRI generates three unique fragments (9.2, 10 and 13 kb), which shows that the EcoRI sites differ. The 17-kb EcoRI fragment generates unique 15-kb XbaI and 10.2-kb EcoRI-XbaI fragments whereas the 14-kb EcoRI locus produces an 11-kb XbaI fragment which contains no EcoRI sites. We also identified the 3' cellular BamHI and SacI sites with the C-p15E and gp70 probes, respectively. Figure 3B summarizes all the data as restriction site maps for the different proviral sites. As it is unlikely that these differences are due to point mutations, because we detect no alterations in the PstI restriction sites within the proviruses (see Fig. 1), we conclude that the cellular sequences around each provirus are different; these new proviruses probably arose through reinfection of germ-line cells and integration at unrelated sites.

We analysed 21 pedigreed mice from the AKR/Jackson pedigree expansion colony in January 1980, to examine the distribution of ecotropic proviruses. Figure 4 shows the relationship between the mice surveyed, outlined as a family tree, and the Southern blot analysis from which we determined whether the mice were heterozygous or homozygous at each locus. The variable bands are more prevalent in this survey than in an original pool of DNA from 20 mice examined in March 1978, when the variable proviruses were present in only 10–20% of the haploid genomes. The 17-kb fragment is present in 22 of



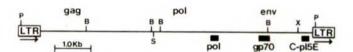


Fig. 1 Individual AKR/J mice, although sibling inbred for over 140 generations, do not carry identical numbers of integrated ecotropic provirus sequences. DNAs from four mice (a, b, c and d) previously identified as exhibiting different homozygous patterns were used in this Southern hybridization. Digestion with EcoRI generates the four different patterns whereas PstI digestion produces the single internal AKV PstI fragment (arrow). The three constant EcoRI fragments are approximately 11, 12 and 50 kb long. The variable proviral EcoRI fragments are 14 kb (lane b) and 17 kb (lane c). Lane d displays all the ecotropic proviral fragments we have detected in AKR/J mice. 10 μ g of purified 15 brain DNA was digested with either EcoRI or PstI. The samples were electrophoresed through a 0.5% agarose gel16 using undigested A phage DNA, A phage DNA digested with HindIII, A phage DNA digested with XhoI, and pBR322 digested with BamHI and Pst1 as size markers. The fractionated DNA was transferred to nitrocellulose filters^{8,17}, hybridized^{17,18} with the gp70 probe, washed¹⁸ and autoradiographed for 10 days with an intensifying screen^{19,20}. A control lane, labelled NIH/Swiss, contained 20 µg of EcoRI digested DNA isolated from the liver of an NIH/Swiss mouse, which does not contain a complete ecotropic provirus21 and shows, as expected, no hybridization. The location of the ecotropic-specific hybridization probes? (black boxes) is shown with respect to a restriction map of the AKV genome¹¹: P, PsrI; B, BamHI; S, Sacl; X, Xbal. The AKV genome contains no EcoRI sites. The long terminal repeats (LTR) are shown as boxes. The probes are single-stranded radiolabelled AKV cDNA restriction fragments7 prepared by the method of D. Schwartz (personal communication). The probes are named according to the gene to which they map. The p15E probe lies within the C-terminal coding region of p15E and is called C-p15E7

the 42 haploid genomes examined and the 14-kb fragment is found in 19. These results are not an average of the entire mouse colony in January 1980, because the mice were selected such that they would be most distant from one another; nonetheless, it is likely that these new proviruses have increased in frequency between the two analyses. The pattern of provirus-containing loci displayed in Fig. 4 indicates that: (1) the common breeding pair at the top of the chart was heterozygous at both loci, (2) there is no obvious linkage relationship between loci and (3) both proviruses probably arose concurrently.

Different AKR substrains carry varying germ-line patterns of ecotropic proviruses in which only the Akv-1 allele is common to all^{6,12}; this suggests that germ-line reintegrations have occurred in these mice and that the original AKR line contained only the Akv-1 locus. Rowe and Kozak¹³ have observed that new virus-inducing loci appear in inbred Akv-1 congeneic mice if the maternal mice are viraemic. These are probably reintegrations of the AKV genome, which suggests that the new loci we

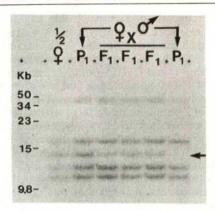
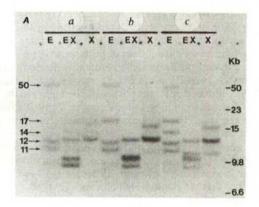


Fig. 2 Southern hybridization analysis of a cross between a male AKR/J mouse lacking the 14-kb proviral EcoRI fragment and a female AKR/J mouse homozygous for this fragment shows that these proviruses are carried in the germ line. The DNA from both parent AKR/J mice was isolated from brain tissue. The F_1 DNA was from 16-day old embryos. In each case, $20\,\mu g$ of EcoRI-digested DNA was loaded in each lane, except the left lane in which only 10 µg of the female parent DNA was loaded. The filters were hybridized with the gp70 probe and autoradiographed for 10 days as described in Fig. 1 legend. The left two lanes contain the female parent DNA, the right lane contains the male parent DNA and the three middle lanes DNA from three individual embryos. The arrow indicates the 14-kb EcoRI fragment which is heterozygous in the embryo genome.



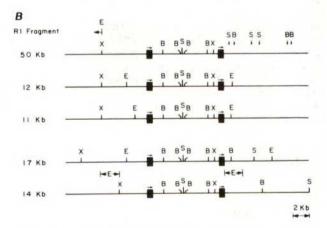
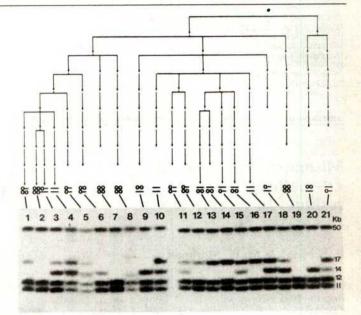


Fig. 3 Digestion of AKR/J DNA with XbaI, which cuts once within the AKV provirus, and EcoRI, shows that the cellular sequences surrounding the ecotropic germ-line proviruses differ. A, Three samples of DNA (10 µg per lane) isolated from the brain tissue of individual AKR/J mice were cut with EcoRI (E) and XbaI (X) or with both enzymes (EX). Sample a contains only the three constant EcoRI fragments, sample b carries in addition the 17-kb EcoRI fragment, and sample c contains all five provirus loci. The filters were hybridized to the pol probe as described in Fig. 1 legend and autoradiographed for 3 days. B, Restriction site maps of the cellular sequences surrounding each of the germ-line AKV proviruses. The provirus sites are from Steffen $et\ al.^{11}$. The $XbaI\ (X)$ and $EcoRI\ (E)$ sites were determined from the data shown in A. The position of the 3' SacI (S) and BamHI (B) sites were determined from similar experiments using the gp70 and C-p15E probes, respectively. We have not discriminated between the 3' cellular BamHI and SacI sites of the three constant proviruses.



Analysis of predigreed mice from the Jackson Laboratory colony reveals the distribution of the variable ecotropic proviruses. A pedigree chart was created for the pedigree expansion colony in January 1980, and 21 mice were selected to represent as wide a population as possible. The origin of each AKR/J mouse is shown in the upper portion of the figure. Each arrow indicates a generation. DNA (20 µg) from each of 21 mice was cut with EcoRI and analysed with the gp70 probe as described in Fig. 1 legend. The interpretation of the autoradiogram is shown schematically above each lane. (0) Signifies the absence and (-) the presence of the variable provirus at a given locus. The filter was autoradiographed for 3 weeks. Samples 4, 5 and 8 contain partial digestion products.

observe in AKR/J mice may arise by reinfection of the embryo

The AKR/J mice seem to be undergoing an amplification of the ecotropic proviruses. Xenotropic viruses might be the result of a past amplification of retroviral genomes. These viruses, which are unable to infect mouse cells but do replicate in cells from other species14, are present in much larger numbers (about 30 proviruses⁷) than the ecotropic proviruses. In a murine ancestor, the 'xenotropic' viruses may have replicated efficiently and amplified their numbers. By natural selection, the murine ancestor may have subsequently become resistant to infection by these viruses, creating a large number of relatively silent endogenous proviruses. Amplification of proviral genomes followed by mutation is one mechanism by which the organism could form a family of related genes, for example, the gp70 family coding for cell-surface markers.

We thank E. Les and Jackson Laboratories for help in screening the AKR/J mouse colony, D. Steffen for the NIH/Swiss DNA, M. Kreitman, N. Lonberg, D. Steffen and R. Weinberg for discussions, and D. Steffen for communicating results before publication. W.H. was supported, in part, by a NSF predoctoral fellowship. This work was supported by NIH grant GM09541-20.

Received 29 December 1981; accepted 22 February 1982.

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Mismatch correction at O⁶-methylguanine residues in E. coli DNA

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Escherichia coli has a correction system which removes mismatched bases from DNA1. Mutants (dam) which lack the major DNA adenine methylase² are hypersensitive to the effects of base analogue mutagens such as 2-aminopurine³ and appear to be defective in mismatch correction. Phenotypic revertants of dam mutants to base analogue resistance include second site mutations in mutL or mutS genes4, which are also part of the correction system. We report here that E. coli dam mutants are also sensitive to the DNA methylating agent N-methyl- N^\prime nitro-N-nitrosoguanidine (MNNG), which introduces O6methylguanine (m6G) into the DNA. This sensitivity, however, was not observed using methylating agents which generate only low amounts of this alkylated base. Furthermore, the introduction of either a mutL or a mutS mutation into dam strains abolished the sensitivity to MNNG. These results suggest that mismatch correction occurs at m6G residues in DNA. These lesions miscode in DNA polymerase I-mediated DNA synthesis in vitro⁵ and are known to be mutagenic in vivo⁶. Nevertheless, it appears that mismatch correction at m6G residues in DNA does not lead to reduced induction of mutation by MNNG.

The major repair pathway for the promutagenic base m⁶G in E. coli is via a methyltransferase induced as part of the adaptive response to alkylating agents⁷⁻⁹. The adaptive response is induced by exposure to sublethal concentrations of alkylating agents and renders cells resistant to the lethal and mutagenic effects of subsequent treatment with such agents9. To allow the maximum possible interaction between m⁶G and the mismatch correction system, except when specifically stated, all the experiments reported were performed in conditions in which the adaptive response is not induced. Figure 1a shows that the dam-3 strain GM113 is more sensitive than its parental strain GM112 (dam⁺) to treatment with MNNG for 5 min. For MNNG, a fairly high proportion (7%) of the total alkylation products are m⁶G (ref. 10). To determine whether the sensitivity of dam strains to MNNG is related to the presence of this base, we tested the sensitivity of GM113 (dam-3) to dimethyl sulphate (DMS) for which m⁶G constitutes only a very minor proportion (0.5%) of the total products¹¹. Figure 1b shows that dam strains are not significantly more sensitive to DMS than dam + strains; this agrees with a previous report that dam strains are only slightly hypersensitive to methylmethanesulphonate (MMS)¹² which also induces only a low proportion of m⁶G residues in DNA.

As the introduction of a mutation into any one of several mutator loci abolishes the sensitivity of dam strains to killing by the base analogue 2-aminopurine^{3,4}, we examined the effect on MNNG sensitivity of these second-site mutations. GM150 (mutL-451 dam-3) and GM169 (mutS-453 dam-3) were more resistant to MNNG treatment than GM113 (dam-3) and were similar in sensitivity to wild-type strains (Fig. 1c). By analogy to the effect of 2-aminopurine on these strains, we suggest that dam strains are sensitive to MNNG because of an attempted mismatch correction at base pairs containing m6G. In the absence of the requisite control normally present in wild-type (dam⁺) strains, this leads to cell death in a high proportion of cases. The introduction of the mut loci prevents the occurrence of this abortive correction mechanism, thus restoring wild-type survival levels. A reduction in the amount of m⁶G in dam strains should therefore result in a decreased frequency of these 'death-prone' repair events and thus enhanced survival after MNNG treatment. This reduction in the level of m⁶G may be brought about by pre-adaptation of the cells. Adapted dam strains should show enhanced survival after challenge with MNNG, but Jeggo et al.13 were unable to demonstrate such adaptation of dam strains to MNNG challenge. However, using a slightly different adaptation protocol, we have demonstrated enhanced survival in these strains after MNNG challenge (Fig. 2a). As normal levels of the m⁶G methyltransferase are induced in GM113 (dam-3) after adaptation (Fig. 2b), we conclude that at least part of the observed adaptation for survival in this strain is due to an enhanced removal of m⁶G.

The abolition of sensitivity to 2-aminopurine by the introduction of a second-site mut mutation into dam strains is accompanied by an increased frequency of both spontaneous and base analogue-induced mutagenesis¹⁴ presumably due to the absence of a mismatch correction system. It was expected, therefore, that MNNG-induced mutagenesis should be higher in the mutL-451 dam-3 strain than in wild-type strains. Table 1 shows that this is not the case. The frequency of spontaneous mutation to rifampicin resistance of GM150 (mutL-451 dam-3) is ~100-200-fold greater than that of wild-type strains as expected. However, the MNNG-induced frequency of mutation to rifampicin resistance is not significantly different between the wildtype strains, AB1157 and GM112, and GM150 (mutL-451 dam-3). GM113 (dam-3) exhibits the smaller but consistent spontaneous mutator phenotype characteristic of dam mutants15 but again is not hypermutable by MNNG. It appears, therefore, that while the relative sensitivity to MNNG and DMS of GM113 (dam-3), GM150 (mutL-451 dam-3) and the parental GM112 (dam+) suggests that mismatch correction does occur in wild-type E. coli at m6G-containing base pairs, this correction system does not reduce miscoding mutagenesis due to this lesion. This interpretation is supported by the data of Sklar and Strauss¹⁶ which indicate that E. coli uvrE mutants, which are also deficient in mismatch correction, are normally mutable by MNNG.

Table 1 MNNG-induced mutation to rifampicin resistance

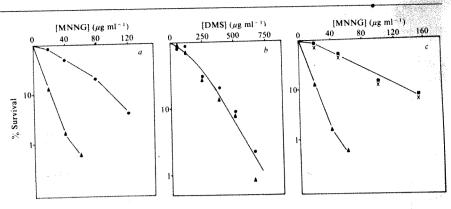
Mut (Rif ^r muta	ation frequents per 10 ⁸	ency bacteria)	
$[MNNG] (\mu g m l^{-1})$	0	5	10	20
Strain				
AB1157 (wild type)	2.6	2,900	4,900	33,000
GM112 (dam ⁺)	1.3	1,100	6,700	25,000
GM113 (dam-3)	94	1,100	3,900	9,600
GM150 (mutL dam-3)	270	2,000	6,200	23,000

Cultures (1 ml) containing $\sim 10^8$ cells ml $^{-1}$ in supplemented minimal medium were treated with MNNG at the concentrations shown at 37 °C for 5 min. After treatment, cells were collected, washed and resuspended at 107 cells ml-1 in supplemented minimal medium and grown at 37 °C for 16-20 h. Appropriate dilutions were plated onto nutrient agar containing rifampicin (100 µg ml⁻¹). Total viable bacteria were determined by plating onto nutrient agar plates. The mutation frequency is expressed as Rif colonies per 10⁸ viable cells.

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Sensitivity of dam-3 strains to alkylating agents. Cultures in the exponential growth stage in minimal salts medium supplemented with 0.5% glucose, 0.1% casamino acids and 0.00003% thiamine were exposed to alkylating agent at the concentrations shown at 37 °C for 5 min. After dilution in 10 mM K phosphate pH 7.0, cells were plated onto nutrient agar. Survival was determined after growth at 37 °C for 24 h. a, MNNG sensitivity of lacktriangle, GM112 (dam^+) and lacktriangle, GM113 (dam-3). b, DMS sensitivity of lacktriangle, GM112 (dam^+) and lacktriangle, GM113 (dam-3). c, MNNG sensitivity of ... GM150 (mutL-451 dam-3); x, GM169 (mutS-453 dam-3); and ▲, GM113 (dam-3).



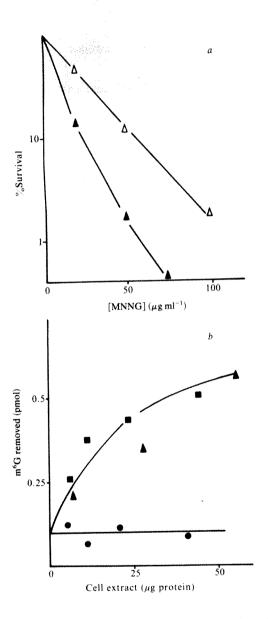


Fig. 2 Effect of adaptation on dam strains. a, MNNG sensitivity. Unadapted cultures (A) were grown and treated as described in Fig. 1 legend. Adapted cultures (\triangle) were grown in medium containing 0.1 µg ml⁻¹ MNNG for 3 h followed by 1 µg ml⁻¹ MNNG for 1 h immediately before treatment. b, Induction of m⁶G methyltransferase activity in adapted dam-3 strains. Adapted and unadapted cultures were grown as described above and then cells were collected, washed and disrupted by sonication. The demethylation of ³H-labelled m⁶G in DNA treated with ³Hmethylnitrosourea was assayed using these crude cell extracts as described elsewhere⁷ ●, GM113 (dam-3) unadapted; ▲, GM113 (dam-3) adapted; , GM1150 (mutL dam-3) adapted

The ineffectiveness of the mismatch correction system in reducing MNNG-induced mutagenesis at m6G may be explained by the presence of the miscoding lesion in the parental DNA strand and by the ambiguous nature of m⁶G coding. After incorporation of 2-aminopurine or bromouracil, the 'incorrect' base remains in the daughter strand. In wild-type cells, the mismatch correction system will recognize the parental strand by its methylation pattern, and will thus always correct the mispair in the correct orientation. However, replication at m6G will direct the incorporation of T or C into daughter DNA. We propose that neither m⁶G:C nor m⁶G:T is a sufficiently good base pair to avoid subsequent recognition by the mismatch correction system. In normal conditions, the mismatch correction system will operate by removing the incorporated T or C, thus allowing a second attempt at the incorporation of the correct base. This second attempt will again result in an imperfect match as no 'correct' base exists. This removal/insertion cycle will presumably continue without check until the dam gene-encoded DNA methylase methylates the GATC sequences in daughter DNA thus removing the strand discrimination necessary for mismatch correction. If the incorporation of C or T opposite m⁶G occurs in approximately the same ratio for the polymerase system involved in replication and in mismatch correction, no net decrease in misincorporation and thus no decrease in mutagenesis will result from the operation of mismatch correction at m6G-containing base pairs.

There are, however, circumstances in which operation of mismatch correction at base pairs containing m6G should be effective in avoiding mutation. If the m6G methyltransferase operates during base removal and replacement and before the action of the dam encoded DNA methylase, the resulting G C or G·T base pair will be either recognizably correct or a true mismatch and dealt with accordingly. In this case, the adaptive response, in addition to its role in removing m⁶G residues before DNA replication, may interact with the mismatch correction system to further reduce mutation frequency.

We thank Dr Michael Green for stimulating discussion throughout this work, and Professor B. A. Bridges and Dr A. R. Lehmann for critical reading of the manuscript. M.G.M. was the recipient of a Faculty Research Award (FRA-149) from the American Cancer Society.

Received 21 September 1981; accepted 25 February 1982.

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MATTERS ARISING

Early hominids and fire at Chesowanja, Kenya

IN addition to providing valuable new information on the important palaeo-anthropological evidence from Chesowanja, Gowlett $et~al.^1$ state that "burnt clay found at one artefact locality dated to greater than 1.42 ± 0.07 Myr is the earliest known evidence of fire associated with a hominid occupation site" and that "the new find, along with the more tentative evidence from other sites, greatly strengthens the hypothesis that by 1.4 Myr hominids were using and controlling fire".

There are various reasons why the evidence reported should be treated as a good deal less definite than the article implies

Traces of bush fires of presumably natural origin are not uncommon in early and middle Pleistocene deposits of the East African Rift Valley. For example, the fluviatile facies of the Upper Member at Koobi Fora contains numerous reddened, hardened patches that are closely analogous to places where smouldering logs and stumps from modern bush fires have burned down against or into contemporary soils. I have seen and photographed several recent examples of burned patches in East Africa, and at least one rather analogous set of circumstances has been reported in Australia². Where these reddened patches, ancient or modern, erode, a scattering of hard red fragments results. Clasts of this natural 'terracotta' can commonly be observed in the grits and gravels formed on ancient land surfaces. I have frequently observed these clasts in excavations both at Koobi Fora and at Olorgesailie and imagine them to be a widespread phenomenon.

This means that before a new record for a high antiquity of human control over fire can fairly be claimed, there must exist some objective means of distinguishing a hearth controlled by hominids from the baking effects of a bush fire. Gowlett et al. seem to claim that this distinction is made possible by the contrast between a determination of a 400 °C baking temperature for the burned material from site GnJi 1/6E compared with over 600 °C for "baking around a recently burned tree stump". I am sceptical that a difference between two isolated determinations can be relied on as a general criterion, and I would predict that if a series of bushfire baked samples is measured, the range of temperatures will be found to overlap with campfire temperatures.

The most convincing evidence for human control over fire in an open site would come from evidence of a localized, burned patch that was not a burned stump, inside the confines of artefactual refuse which had not been unduly disturbed by fluvial transport³. However, paragraph 4 and Fig. 2 of the article¹ make it clear that all the finds, including the burned earth fragments, were recovered from material that was swept together in the bed of a small channel. The finds can thus be regarded as provocative and suggestive, but no more.

The question of the antiquity of human control over fire is of more than curiosity value in our understanding of prehistory and human evolution. Besides the indication provided of an advance in mental capacity, fire may well have had important effects on feeding strategies and diet breadth. Control over fire also would have allowed humans to raise the frequency of bush fires and thereby to have a marked effect on vegetation patterns.

The article by Gowlett et al., and others that they cite, serve the useful purpose of emphasizing that we still do not know whether humans controlled fire before ~0.5 Myr and if so, how long before. I suspect that development of suitable discriminants between traces of bush fires and controlled fire will require collaboration of physicists and archaeologists in the field during excavations.

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GOWLETT, HARRIS AND WOOD REPLY—Isaac provides us with an opportunity to explain further why we consider that the context of the burnt clay at site GnJi 1/6E deserves serious consideration as evidence linking fire with hominid activity.

Isaac describes a series of circumstances which would provide the least ambiguous evidence of controlled fire on a single open site, and few would dispute his general criteria. We agree, too, that circumstances where baking is not proved, or where evidence of hominid activity is tenuous, provide inadequate testimony. Where we clearly disagree with Isaac is over the idea that a site with much stronger evidence should have no effect on what can be admitted as a working hypothesis.

We emphasize again that the evidence of fire at Chesowanja is quite definite: the clay was burned, and its association with the artefacts is direct and physical. Isaac rightly points out that the apparently low baking temperature of the clay is inconclusive evidence of the nature of the fire. This we clearly acknowledged, and it is why we advocated studies of the cooling

rate of the clay. We share with Isaac the hope that future investigations of experimental fires will assist in the interpretation of such data.

Strength of association is an important point. Given the controversy surrounding the contexts of all early sites1, Isaac legitimately raises the possibility that the baked clay and artefacts may have been swept together. The evidence does not, however, support this view. On the contrary, we have good reason to believe that a reverse process has operated, and that the clay lumps, starting together, have crept apart. The presence of several large lumps together (square 82-83 E/111-112 S), including three weighing 262, 217 and 175 g respectively and which retain sharp edges and protuberances, argues very strongly against any significant water transport, as indeed does the whole context of fine-grained sediments. These finds occur close to a modern erosion gully, which prevents further exploration to the north and west, so that the scatter of smaller clay lumps to the opposite corner of the excavation is actually a vital bonus to interpretation, as it rules out any possibility of the burnt material deriving from recent superficial disturbance.

We welcome Isaac's contribution, for it is important that the cases for and against early human control of fire, at Chesowanja or elsewhere, should be fully aired. We also agree that progress in this field of research will be much facilitated by closer practical collaboration between physicists and archaeologists. Nonetheless, we stand firmly by the view that the Chesowanja evidence at strengthens the hypothesis that fire was associated with hominid activities more than 1 Myr ago. We shall present additional evidence in due course.

We hope that this exchange will help to stimulate research on a wider scale, for although the evolutionary importance of controlled fire has been appreciated for over a century², new technical means for exploring its history now provide increasingly better chances of adding to the facts.

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BOOK REVIEWS

Rolled over by technology?

Bruce Williams

THE God that limped? Hephaestus, the Greek god of fire and metalworking, was eagerly sought after for his technological skills. But he was ugly, irascible, often disliked by the other gods, and he had a pronounced limp. For Colin Norman this makes Hephaestus the appropriate symbol for modern technology — powerful and versatile but often marred by crippling defects.

A.N. Whitehead once wrote that the greatest invention of the nineteenth century was the invention of the art of invention. During the Second World War, J.A. Schumpeter predicted that industrial innovation would become a routine function of organized R & D. Planned inventions of weapons during the War, and a great range of planned inventions after it - sputnik, rockets to put men on the Moon and bring them back again, computers, chips, a great range of pesticides and ethical drugs, high-yield grains to create the green revolution encouraged many to believe that the twentieth century had invented the science of invention. But that euphoria was undermined by the extent of serious problems created or intensified by the massive increases in R & D activities (the unforeseen side effects of new drugs, radioactive wastes, the displacement of labour), by fears that some of the solutions are clever but ephemeral (boosting crop yields from oil-based fertilizers), and by growing doubts about the capacity of R & D to contribute much to urban decay, poverty in the Third World, de-skilling, technological unemployment and the exhaustion of non-renewable resources.

While recognizing the immense social, economic and cultural benefits that science and technology have brought to society, Norman concentrates more on the social and environmental costs in the global economy. This is because he judged that present policies and trends are dangerous - the great post-War boom was built on an unstable foundation of cheap oil, and even now R & D in North America is more suited to the military needs of the 1950s than to the social needs of the 1980s. Whether the mismatch between defence and social needs in R & D objectives is any greater now than in the 1950s is not explored but is implied in his comments on the energy crisis and the problems of producing sufficient food, shelter and material resources for a population that will swell to six billion by the year 2000.

For those directly involved, there has

The God That Limps: Science and Technology in the Eighties. By Colin Norman. Pp.224. ISBN 0-393-01504-1. (W.W. Norton: 1982.) £10.50, \$18.25.

always been an element of fear associated with the introduction of new technologies. Norman refers to the growth of a more general fear that social changes are determined by technological change that is driven chiefly by its own momentum -"we even speak of new generations of computers, automobiles and other high technology goods as if they were biological descendants of earlier models". He explains these growing fears in terms of the complexity of many modern technologies and the centralization of decision-making by both Governments and large corporations. On "the fear that society is simply a product of its technology", he points to the major part that Governments play in determining the extent and directions of R & D and the numbers of scientists and engineers, and to the vast amount of innovation in, for example, weapons, space vehicles, law enforcement technologies, health care systems, as well as to the research activities in astronomy, high-energy physics and molecular biology, that cannot be explained even in terms of economic forces. Technological, economic and political forces interact to produce a complex array of pressures that push and pull technological developments along certain paths. The technical imperatives only define what is possible.

How, then, may technological development be pushed and pulled on to the path leading to a humane and sustainable society? He suggests four things - first, a better balance between unfettered market forces and Government constraints on market forces to reduce social costs; second, a broader public participation in the decisions that lead to the generation and adoption of new technology to give those most likely to be affected a role in its planning and to reduce some of the negative impacts; third, a greater access to modern technology in developing countries, and on more favourable terms, and an increase in their capacity to generate and apply technologies most suited to their own needs and resources; and fourth, a recognition that technology cannot by itself solve social and political problems.

His first suggestion is based on the judgement that the unfettered workings of the market system are enormously powerful in stimulating the development

and application of some technologies but cannot always be relied on to steer technological developments along socially appropriate paths. There is therefore a need for Governments to establish certain constraints - on pollution and working conditions - and to supplement market forces by subsidies for the production of certain commodities or services (including R & D). Government constraints on and supplements to market forces have been established for many years, the fact that competition is a form of social control based on legislation has been firmly accepted, and the theory of how to use taxes and bounties to control market forces when private and public costs or benefits diverge was established by Pigou 50 years ago. That Governments have not taken the appropriate actions - and Norman recognizes that some of their regulatory actions (on, for example, oil and gas prices in the USA) have made things worse - is sometimes due to the power of special interests, sometimes to unwisdom, and sometimes to the inability of scientists or engineers to provide firm evidence on the costs and benefits of various proposals for regulatory action. Norman writes of the urgent need for regulatory reform but his account of just what should be done and how is too cursory.

Norman admits that the experience of public participation in decision-making on new technologies is limited, but he is optimistic that it would have a beneficial effect on technological development and reduce some of the negative impacts.

He gives some examples of effective public participation in decisions in the USA, for example the Alaska Pipeline and the SST, and it is reasonable to demand and expect improved environmental impact statements and public discussions of them. But he underestimates the problems of participation. With new printing methods, for example, at what stages in the development of several technologies could there have been "public participation"? And when the publishers decide to use the labour-saving new technology who is to be "the public" to participate in the decisions? The new technologies that affect an industry may be developed and established outside that industry or in another country. The "compulsions of competition" are very complex, the effects are frequently impossible to foresee and there is need for much more consideration of how to distribute the costs of technical change.

His suggestions for providing the Third World with technology on better terms, for cooperation with the developing countries in the transfer and adaptation of technologies, and for the indigenous development of appropriate technologies are more positive and complete.

The God That Limps is a timely and stimulating book. In warning of the need to recognize that technology cannot of itself

solve social and political problems Colin Norman allows a much greater role for technology than Jay Forrester, who recently asked whether for the past 200 years technology has done much more than cope with the consequences of population growth.

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Meteorology enters the mesoscale era

Edward J.Zipser

Meso-scale Atmospheric Circulations. By B. W. Atkinson. Pp.495. ISBN 0-12-065960-3. (Academic: 1981.) £32.40, \$78.

FIFTY years hence, it may be difficult to explain to students how most meteorologists contrived to postpone to the 1980s their discovery of the importance of the mesoscale, the intermediate scales of motion. Indeed, how could so many observers of the natural world have accepted for so long that local weather events were controlled by large-scale systems with a spectral gap in the mesoscale (tens to hundreds of kilometres)? While better observations and numerical models have improved forecasts on time scales of 1-3 days, our ability to forecast 1-12 hours into the future, or to anticipate severe weather or flooding rains, has stagnated for 20 years.

Atkinson has written the first book in mesoscale meteorology, and his pioneering effort sets a high standard. He has made eminently reasonable selections of what to include and what to exclude. Each subject is treated in a similar manner, with observational knowledge summarized first, followed by theories, numerical simulation results and, where applicable, hardware models.

Many of the subjects have never been summarized comprehensively before. The first half of the book is a superb, systematic review of topographically induced flows, including lee waves, downslope winds, sea-and land-breeze circulations, and slope and valley wind circulations. The literature has been surveyed exhaustively; numerous papers hitherto unknown to this reviewer are placed in context and, where appropriate, recent papers highlighted. It will be difficult to improve upon this treatment, and Atkinson's book could well become the classic reference work on these subjects for the next 20 years.

Severe local storms have been the centrepiece of mesoscale meteorology for many years. Storms which generate hail, tornadoes and other damaging winds are challenging subjects because they are intrinsically scale-interaction phenomena. The severe storms chapter is excellent on convective storms in general, and in

summarizing recent literature on hailstorms, but it is rather weak on the rotational aspects and on recent literature on tornadic storms. The recent work on squall lines is well-covered, but it should have been clearly separated from the severe storms chapter and placed in a section on mesoscale convective systems, which are larger than individual storms. By not distinguishing between organized convection and severe convection, as where squall lines are claimed to be severe by definition, Atkinson's book will help some long-held misconceptions to persist for a few more years.

After a useful chapter largely concerned with recent results on shallow cellular circulations, the book closes by treating mesoscale circulations within cyclones. Atkinson himself has made contributions to extratropical cyclone studies, and this section is strong. Because this type of research concentrates upon radar description of precipitation distribution, however, it may not be obvious to all readers when it is proper to make inferences about mesoscale wind circulations in the absence of direct measurements. In addition, the section on tropical cyclones, like the literature on which it is based, is somewhat uneven. The recent improvement in aircraft instrumentation should lead to the need to undate this section in the near future. For unknown reasons, no mention is made of cyclonic circulations in the tropics other than tropical storms.

This book does not attempt to be all things to all people; it deals with basic science, not applied science. The sea breeze, for example, is treated as a basic fluid flow, and the importance of that flow for precipitation distribution and air quality, although mentioned, is covered either briefly or not at all.

The author can take pride in fulfilling a long-standing need for a text in mesoscale meteorology, which is sure to stand as a basic reference work for decades. Nearly all meteorologists will want a copy close at hand.

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People and change

William Brass

Population and Technology. By Ester Boserup. Pp.225. UK ISBN 0-631-12817-4 (Basil Blackwell: 1981); US ISBN 0-226-06673-8 (University of Chicago Press: 1981.) £12.50, \$17.50.

In the early 1960s Ester Boserup began to propagate the view that population growth was one of the fundamental causes of the development of new agricultural methods. The importance of her work was less in the idea than in the lucidity and coherence of the demonstration. The present book is a wider essay on a similar theme, the interaction of population growth and technological change.

The central idea is that population factors have been a major determinant of the introduction of (or failure to exploit) new methods for the organization and improvement of production. Since the scope of the book is the whole of human history, much of it is logically concerned with agricultural output. The presentation is not of a highly structured argument from the idea to the consequences; rather there is an extensive historical review beginning with the transition from hunting-gathering to agriculture, and ending with the population-technological relationships in the less-developed countries of the present day. In between there are accounts of the establishment of urban civilizations, with particularly interesting sections on Mesopotamia, the Maya and China, the development of trade and industry in Europe, mass-migration to the United States and elsewhere, the transfer of industrial technologies to Asia and much else. Despite the unifying theme there is no dogmatism; population growth may be taken as the driving force but the importance of effects in the reverse direction is implicitly accepted.

Again and again in traditional works of pre-history and history there is a sentence which states that as a consequence of technological change (settled agriculture, spread of the use of iron, industrial invention) the population grew. There is seldom even a cursory consideration of how this happened; the way in which the changes could have altered fertility and/or mortality appropriately is left obscure. The large increase in the knowledge of the basic demography of less-developed communities in recent years has intensified the difficulty of the explanation. No simple, universal link between technological development and reduced mortality or increased fertility can be demonstrated.

The God that Limps: Science and Technology in the Eighties, by Colin Norman (reviewed on page 871) will be published in Great Britain on 26 May 1982.

The turn-around in the direction of the pressures, proposed by Ester Boserup, is stimulating and attractive although it leaves the causes of population growth in darkness, almost as a natural law of human history.

In a relatively short book of such a wide scope the evidence to support the thesis is inevitably condensed and the statements sweeping. Readers with a specialist knowledge of particular topics are likely to find assessments which do not take account of the most up-to-date research; the publications relied upon are not always the most reputable. For example, the discussions of the basic demography of the biosocial determinants of fertility and mortality, which are of critical significance for the reversal of the older views on the direction

of the relation between population growth and technological change, are surprisingly weak in depth of knowledge and citations of relevant research. Nevertheless they are sensible and only marginally misleading.

This is an exciting book, full of ideas which are provocative because they are unconventional and yet plausible. One of many possible examples is the examination of the minimum population densities needed for the development of urbanization at the relevant stage of technology. There is undoubtedly much to criticize in detail, but only by studies of this kind can the essential broad syntheses be achieved.

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The doctor of revolution as letter writer

Gavin Bridson

The Letters of Erasmus Darwin. Edited by Desmond King-Hele. Pp.363. ISBN 0-521-23706-8. (Cambridge University Press: 1981.) £45. \$95.

THAT remarkable polymath, Dr Darwin, has been singularly fortunate in having such a loyal admirer in Desmond King-Hele who has stayed beside his hero for some 20 years. His introduction to Erasmus Darwin (1963), followed by The Essential Writings (1968), Doctor of Revolution (1977), introductions to reprints of Darwin's Botanic Garden and The Temple of Nature (1973), and articles in Nature and elsewhere, all demonstrate this loyalty. His high regard for the amiable, versatile and near perfect eighteenth-century genius is no secret. In Doctor of Revolution he declared

Though I may be biased, I regard Erasmus Darwin as the greatest Englishman of the eighteenth century. If you disagree, can you name anyone else in the past 250 years with a list of accomplishments so numerous, so notable and so varied?

These he summarized in a list of 75 achievements which included such unsuspected topics as abolition of slavery, air travel, biological pest control, copying machines, oil drilling, rocket motors, speaking-machines, submarines, water closets and women's lib. He optimistically added that

The flow of specialised articles in journals has been increasing, and, if the momentum continues, we may yet see Darwin receiving the kind of scholarly attention that has been lavished on lesser figures like Boswell or Horace Walpole.

A glance at the *Isis* bibliographies shows that the flow has not been so great and that King-Hele's writings still provide a major

tributary. Little surprise, then, that he himself should edit Darwin's letters.

After a wide search for manuscripts in Britain and the United States, King-Hele believes that he has located the great majority of surviving letters and that this 'provides a balanced and reasonably complete picture of Darwin the letterwriter in all his moods". For a man whose published writings ran to a million and a half words it is disappointing to find that the picture is composed of only 272 examples written between 1749 and 1802. No guess is made as to the likely quantity that may once have existed. We find that Boulton, Watt and Wedgwood kept 103 letters between them, and his grandson, Charles, quoted from 30 out of a collection that has not survived in manuscript. We must be grateful that as many as 88 other recipients make up the rest, that they spread over all but a handful of Darwin's. 50 or so adult years, and that they reflect so much of the man and his achievements for about half of King-Hele's list of 75 are apparently touched upon here. 187 letters survive in manuscript (162 in Darwin's own hand) and the rest are taken from published sources.

King-Hele has developed a meticulous editorial apparatus for the treatment of the chronologically presented sequence. Each letter is printed without the inclusion of any editorial distractions, according to his stated rules for transcription. Passages of some length are omitted in a few instances where these amount to an inserted "paper", such as details of Darwin's polygraphic machine and instructions on landscape drawing which have been removed from two 1779 letters. However, it is particularly pleasing to have facsimiles of the numerous sketches by means of which Darwin illuminated his observations, ideas and inventions.

The editor's notes fill the spaces between the letters, and fill they sometimes do - 18 separate notes on a letter to Watt, and 100 lines of small-type notes on a 12-line letter to Joseph Banks. With a modest number to work on. King-Hele has been able to examine each letter under a powerful lens and reveal minutiae that might escape even the attentive reader's eye. Twenty years' research have brought him to an intimacy with his subject that could scarcely be equalled on the basis of surviving records, contemporary comment and astute interpretation. A dazzling breadth of erudition is woven into the fabric of his scores of enthusiastic and revealing notes. No stone, it seems, remains unturned and one admires his ability to meet the demands that the wealth of Darwin's ideas and activities has placed on him.

Precise information on the source, previous printings and text used for transcription, accompanies each letter, together with notes on the recipient, the date of the letter, explanations of obscurities and identification of people, places and things. A handbook on Darwin's life and times could be assembled from such detailed, well informed and bibliographically replete commentaries. Their biographical content alone is remarkable, for which the assistance of Hugh Torrens is acknowledged.

Fortunately for the reader, studiously careful guidance to all this scholarship is provided by means of name and subject indexes, a chronology, list of recipients and a gallery of 48 portraits, all the product of much thought and time. In fact the body of the text is so replete with editorial matter that it makes for slow reading if one progresses through letters and notes in turn.

As we have come to know from many previously published extracts, Darwin's letters are extremely engaging and entertaining reading, vividly reflecting his moods, interests, personal relations and doings. One really must read through runs of letters by themselves to get their flavour and then retrace one's steps for the additional pleasure of King-Hele's commentaries. Some of the letters are frankly dull, dealing with pedestrian business matters, and but for the overall scarcity of his correspondence might not have been included or accorded such reverential treatment. But most are packed with interest, varying from light-hearted banter to grave formality, and including verse, poignantly expressed sentiments, highly detailed technical descriptions. comments of immense foresight and wisdom, and reflections of his endless vigour, excitement with life and overwhelming good nature — in short, all that his biographers have taught us to expect.

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The what, why and wherefore of cells

R.E. Stephens

Ultrastructure, Macromolecules, and Evolution. By Lawrence S. Dillon. Pp. 708. ISBN 0-306-40528-8. (Plenum: 1981.) £43.79, \$69.50.

IN THIS second part of an intended trilogy, Lawrence Dillon interweaves discussion of evolutionary and cell structural relationships in order to consider genetic mechanisms working at the cellular level and to reconsider phylogeny from a cellular viewpoint. This is a formidable task because of the breadth and depth of information which must be assembled, assimilated and analysed. In terms of defining general principles, Dillon succeeds admirably; in terms of accurately documenting his background data, he fails in certain areas. Most of these failures are inconsequential to the ultimate conclusions, but they are disconcerting nevertheless.

The author has arranged his material into four areas of comparative cytology: cell motility, secretory organelles, energyprocessing organelles, and karyo- and cytokinesis. He begins with a detailed account of the structural chemistry of membranes in general, giving an excellent historical perspective. Oddly, he follows this with four somewhat uneven chapters on cell motility before returning again to membrane-based systems. Motility is covered in chapters on microtubules and microfilaments, cytoplasmic movement, the flagellum and the basal apparatus. There are some serious problems in this area, to which I will return below.

Secretory systems are dealt with in two chapters — the endoplasmic reticulum and the Golgi apparatus are treated as a continuum, the "endomembrane system", while the lysosome, peroxisome and related structures are dealt with as the "vesicular system". This manner of presentation is quite informative in a comparative sense and it is logically and clearly arranged.

Three closely-correlated chapters are devoted to "energy-oriented organelles". The first discusses general aspects of cell respiration and includes a lucid consideration of the evolution of the tricarboxylic acid cycle; the remaining two cover mitochondria and chloroplasts, their comparative structure, replication and development in numerous organisms. Here, Dillon presents a well-balanced account of the two contending theories for the phylogenetic origin of these organelles: the endosymbiotic concept and the episome theory. He clearly favours the latter and presents additional evidence for it, based mainly upon some compelling evolutionary considerations.

Dillon ends this work with a comparison of both nuclear and cytoplasmic division

mechanisms in a multitude of organisms. Here, considering the abundance of information now available, I would have liked to see further examples, mention of odd exceptions and more detail, but the coverage is certainly adequate for the author's purposes. Tacked on to this final chapter are Dillon's two principal conclusions, concerning the inadequacy of simple mechanistic theory (i.e. the central dogma and self-assembly) to account for the development of cell structures and his well-stated contention that the phylogenetic stages of evolution, examined in terms of cellular evolution, allow no clear division between plants and animals. protozoans and algae, or even prokaryotes and eukaryotes. Certainly not everyone will agree with his views, but he raises many points that cannot be ignored.

The problems in the motility chapters. mentioned previously, range from simple inaccuracy (outer doublet B-subfibres contain 10 or 11 protofilaments, not 9; p.73 and p.143) and typographic atrocities (actinomysin for actinomycin, p.255; actinomyosin for actomyosin, p.407), to gross errors in reporting published conclusions (no analysis of doublet A and B tubulin components ever showed a specific number of residue differences, p.174) and unwarranted generalizations (in only one species has the ciliary membrane been shown to differ from the flagellar membrane, p. 163). Also there are mistakes and misjudgements in citation priority, attributing the right facts to the wrong workers, and making overly broad generalizations from authors' more conservative conclusions. These, however, are no more than minor flaws which may easily be corrected in a second edition.

Perhaps more seriously, the dust jacket promises a new concept of flagellar movement. Dr Dillon argues against the widely-accepted sliding filament theory. concluding that it simply isn't so because microtubules are attached to the basal body. He begins his arguments by not mentioning Peter Satir's classic work on the subject, and by not considering that sliding is local and constrained and that the axoneme may twist in response to sliding. He concludes that the tubules themselves must change length, ignoring the work of D. P. Costello who argued all of this a decade ago and did so far more convincingly. The book would be much better off without this "new concept", particularly since it has nothing to do with Dillon's major premise.

Chemical differences in tubulins are central to Dillon's prime example for the workings of the cellular genetic mechanism (pp.174-176; p.558). With a simple self-assembly mechanism, how do the tubulins of the outer doublets and central pair sort

themselves out from each other and from cytoplasmic tubulin and, furthermore, when assembled into a 9 + 2 structure, how are dynein, nexin and spoke sites specified? The author contends that the mechanisms must be enzymatic. If the tubulins are really as different as Dillon implies, they should be able to sort themselves out easily (specific recognition sites) and also to specify binding sites when assembled (differences in surface lattice). In reality, however, the tubulins are not very different and some observed differences are probably due to post-translational modification, perhaps even after assembly. The truth of the mechanisms probably lies somewhere between these two viewpoints. This potentially important example sorely lacks authority and is likely to mislead the casual reader.

As an evolutionary exposition, Ultrastructure, Macromolecules, and Evolution is unique; nowhere is such a broad sweep presented so succinctly in a single source. The references, cited with full title and grouped by chapter at the end of the book, are quite valuable in their own right. This will be a useful reference book for those interested in comparative ultrastructure and certain phylogenetic aspects of biochemistry, and could also serve as a text for a graduate-level cell structure—function—evolution course, leading students easily back to the original source material.

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The healing way

N.R. Rowell

Tissue Repair and Regeneration. Handbook of Inflammation, Vol.3. Edited by L.E. Glynn. Pp.579. ISBN 0-444-80278-9. (Elsevier/North-Holland Biomedical: 1981.) \$136.50, Dfl.280.

REPAIR is essential to life, but what governs whether the body replaces damaged or dead tissue by tissue indistinguishable from the original, by scar or by a mixture of both, is a mystery. A distinguished international group of contributors here present a series of reviews concerning certain aspects of this problem. It is the third of five volumes dealing with inflammation, the first two of which dealt with chemical messengers and cell biology.

In the opening chapter Gabbiana and Rungger-Brändle discuss the fibroblast, the fundamental cell in repair. From work in human beings and experimental animals, this cell has been shown to be remarkably adaptable and may after its morphological, biochemical functional features. In wound healing, fibroblasts, and possibly other cells, transform into contractile cells called myelofibroblasts and these are probably concerned in wound contraction. Unfortunately, the authors cannot yet identify the factors controlling this process. Fibroblasts are also involved in the synthesis and metabolism of connective tissue, including collagen. Collagen has several functions as well as its primary role as a support. Duance and Bailey describe the five known types of collagen but admit that the relationship between structure and function is unknown. As many as 15 cell types may be involved in wound healing, including cells derived from the bone marrow, thymus, reticuloendothelial system and liver, as well as the cells of the tissues involved.

In regeneration, growth-regulating factors encourage the tissue to replace damaged areas with normal-functioning cells. Usually the formation of scar tissue is preceded by physical or other trauma, but this is not always the case; for example, in Dupuytren's contracture and scleroderma trauma is absent.

The book is nicely balanced between a consideration of basic processes and how these apply in the skin, central nervous system, lungs, heart and kidney. The editor himself has written an excellent chapter on the pathology of scar tissue, which links several of the authors' contributions. All authors agree that hyperplasia and atrophy, regeneration and repair are extremely complex phenomena, but in this book they have generally succeeded in making the subject intelligible to their readers.

Each chapter includes between 200 and 500 references which indicates the coverage of the subject. It also probably accounts for the fact that there are few references after 1978, although in one chapter additional notes have been added in 1980.

I can recommend this excellent book; it will be very useful for medical and non-medical scientists in many disciplines. Considering the complexity of the subject, the book is easy to read — presumably the result of sound editing — and there are few mistakes of any note and little overlap between contributions. Further volumes on the immunology of inflammation and the molecular basis for anti-inflammatory therapy will round off the series, which, when complete, should prove a most valuable addition to medical libraries in particular.

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Modelling the growth of literature

B.C. Vickery

Scientific Information Systems and the Principle of Selectivity. By W. Goffman and K.S. Warren. Pp.191. ISBN 0-03-056081-0. (Praeger/Holt-Saunders: 1980.) £13.75, \$19.95.

IMPRESSED by the ever-growing rate of scientific publication, the authors of this book decided that the process of scientific communication needed to be studied as an "ecosystem". They develop mathematical model for the growth of the literature in a defined subject, and then go on to a detailed quantitative analysis of various bodies of literature. The main experimental data consist of a bibliography of schistosomiasis, covering the period 1852-1962 and comprising over 10,000 references. There is little use made of the mathematical model in subsequent analysis, and there is no convincing demonstration of its validity.

Six kinds of analysis were made: distribution of articles and authors by language and by date; multiple publication by individual authors; multiple authorship of articles; most prolific journal titles and distribution of articles among journals; similar analyses for sub-areas of schistosomiasis research; and movement of researchers among sub-areas. The most interesting of the analyses is that based on co-authorship of articles. If authors A and B collaborate, also B and C, as well as C and D, then A,B,C,D can be said to form a network. Of the 6,500 authors in the schistosomiasis bibliography, 2,300 had no collaborators, but there was one big network that embraced 1,750 authors, as well as several hundred smaller networks.

This network of co-authorship is the only real evidence of scientific communication that is examined in the book. The citation of one author by another (implying awareness and probable reading of another's work) is not explored, still less any other forms of communication (attendance at conferences, for example).

The authors then conducted a qualitative evaluation of the schistosomiasis literature. Some 47 active workers in this subject were chosen with the aid of expert advisers. Each was asked to examine the bibliography and indicate "articles you deem of lasting importance". Over 3,000 of the 10,000 articles were selected at least once; about 1,600 at least twice; 471 at least five times; and 69 papers were selected 12 times or more. As one moved from the 3,000 to the 69, the likelihood of the authors being members of the large coauthorship network not surprisingly increased from 40 per cent to 70 per cent.

Goffman and Warren are convinced that "quality filtering" of scientific literature is needed in order to ease the task of retrieval, and offer proposals based on citation in review papers as an indicator of quality. I

find it doubtful whether any further mechanism — other than the review papers themselves — is needed by the scientific community.

The book presents a number of interesting ideas about the structure of scientific literature, and Goffman and Warren certainly extract as much as they can from the data used. But this information simply consists of the authors, title and source of each article, plus its evaluation by experts. There is no indication of what the authors have read, or who reads their contribution. In order to get a more rounded understanding of scientific communication, the book needs to be complemented by the reading of work such as that of Garvey, who has taken these other factors into consideration.

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The complete angler

Bruce R. Moore

Circular Statistics in Biology. By Edward Batschelet. Pp.372. ISBN 0-12-081050-6. (Academic: 1981.) £28.80, \$69.50.

MANY scientists who rarely otherwise cite secondary sources, do so routinely when referring to statistical tests. Several reasons for this tradition are evident, not the least of which is that original statistics papers are meant to be read by statisticians.

This use of secondary sources is frequently quite harmless, since clear and accurate statistics texts are available at many levels. It has sometimes been less satisfactory, however, for that substantial minority of scientists whose data occur in vector form. The *t*-tests and ANOVAs of everyday statistics are seldom applicable to angular data. A number of special tests have, in fact, been developed, but they are not described in most statistics texts and are therefore missed by many potential users. In consequence, statistical inference based upon mere intuition has become the norm in certain areas.

Against this background, one finds isolated regions where the special tests have been thoroughly exploited. The source most often cited in these areas is an out-of-print monograph by Edward Batschelet (American Institute of Biological Sciences, 1965). Although extremely specialized, this work has become for many readers not only a highly trusted source but even an object of personal affection. Such persons were pleased to learn in recent years that, while approaching retirement, Professor

Batschelet was preparing a book-length expansion of the monograph.

Although he died unexpectedly a few months after retirement, Professor Batschelet's manuscript had been completed, and the book has now been published. Readers will find in it the same clarity of style and careful choice of example which characterized the earlier monograph. Essentially, Batschelet describes some 30 tests for vector clustering, group differences, correlations and goodness-of-fit. Most of these are briefly explained, then illustrated with one or more examples. Few derivations are given. The examples are taken almost entirely from biology, but readers from other disciplines should find them clear and easily translatable. Only slightly less clear are the supporting chapters describing various circular distributions and simple mathematical techniques.

There are a few mistakes: Example 6.5.1 requires k = 80; 7.4.1, $a_s = 2.25$; 7.7.1, $T^2 = 154$; p.183, Σ sines = -1.48; and Table 9.3.2, a fresh beginning. The two-stage application of Mardia and Watson-

Wheeler tests in 7.8 is appropriate, but the sanctioned alternative combination of Mardia and Watson-Williams is not. The choice of median peak hour in 1.5.2 is not altogether proper, nor is the "careful" breaking of ties recommended in 9.3. But a few such lapses are almost inevitable; while unfortunate, they detract very little from the value of the book.

Circular Statistics should prove extremely helpful to almost anyone with vector data. It can be recommended also to potential writers of more general statistics texts. A worthwhile precedent is being set here by J. Zar's Biostatistical Analysis, the second edition of which, shortly to be published by Prentice Hall, includes two full chapters on vector-statistical methods. Batschelet offers an excellent starting point for a generation of other such chapters.

Altogether, we have received a most valuable legacy in this long-awaited and authoritative work.

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Life viewed in the particular

B. C. Goodwin

The Foundations of Biological Theory. By E. H. Mercer. Pp.242. ISBN 0-471-08797-1 (Wiley: 1981.) £29.20, \$22.55.

A PERENNIAL source of tension in science arises from the possibility of explaining phenomena in two distinct ways: either as a result of law, whereby they become intelligible in terms of principles at once simpler and more general than the phenomena themselves; or as a result of contingencies, of events that just happened to occur. A classic example of the latter was Buffon's suggestion that our planetary system arose from a chance event, a nearcollision of the primitive Sun with another celestial body. Kant, on the other hand, suggested that orbiting planets arise by necessity (i.e. law) from gravitational instabilities in rotating masses of gas. Buffon the biologist chose a description in terms of particulars; Kant the philosopher sought a decription in terms of universals. Current theory favours Kant, with farreaching consequences, among them the expected existence of countless other planetary systems in the cosmos with conditions allowing life to evolve.

Biologists still favour description in terms of particulars, and this view dominates E. H. Mercer's clearly-written book on the conceptual foundations of biological theory. He assumes that all biological phenomena are instances of physical and chemical laws, but because of hereditary mechanisms, certain processes that arise by chance in organisms can be perpetuated so that organisms become the sediments of historical contingencies which constrain in adaptively successful ways physical and chemical possibilities. For him "the historical course of events leading up to the present situation . . . provides biology with its theory". The task of theoretical biology is thus to tell the story of evolution, a point of view prevalent since Darwin. This has the important consequence that the biological realm is not rationally intelligible in the sense of revealing the operation of universal principles of organization and transformation. Life on other planets could look very different.

Mercer bases his analysis on the important distinction, established in Newtonian physics, between universal laws embodied in differential equations and the particular conditions which define a unique solution for a specific process. Although "boundary conditions" usually refer to particulars for fields, Mercer uses this term to include initial conditions, and he extends it to the more general concept of constraint, which simply imposes some limitation on the system. His treatment of biological organization is then presented in terms of a hierarchy of constraints which he describes as "organising or operational rules that define functional relationships between the units and regulate their interactions". The goal or purpose of biological organization is, he assumes, to ensure its survival. This is an extrinsic stability criterion.

Many of the arguments are already familiar from the writing of Polanyi, Pattee, Riedl and others, to whom Mercer refers. Relevant ancillary concepts from such areas as information and control theory, thermodynamics and kinetics are clearly described, and these ideas are then used to describe organismic processes in terms of a hierarchy of constraints, with descriptive detail from biochemistry, genetics and development. A more analytical treatment in terms of nonequilibrium thermodynamics is less successful since Mercer claims that the near-equilibrium theory gives satisfactory account of the dynamic stability exhibited by adult organisms". However, such periodicities as circadian rhythms, neural pacemakers and peristaltic rhythms are excluded from this domain. Also, he incorrectly implies that a thermodynamic characterization has been given of these and other far-fromequilibrium phenomena; what has been obtained is useful insights into non-linear behaviour and symmetry-breaking by a number of investigators using stability and bifurcation theory, some of which Mercer describes.

Chance plays the conventional role of generating the variety on which natural selection operates, and the various sources from quantum indeterminacy to macroscopic uncertainty in bifurcations of "chaotic systems" and chromosome crossing-over are described. A final chapter outlining the origin and evolution of animate systems, continuous with physical evolution, completes the work. We are thus provided with a detailed account of organisms as constrained physico-chemical survival machines, both their hierarchical structure and their variety arising by chance.

Mercer's book has the virtues of clarity and extensive scholarship, only occasionally flawed by an incomplete grasp of subject matter. It provides an up-to-date account of neo-Darwinist thinking dominated by the "evolutionary paradigm", which defines the prevalent conceptual tradition in contemporary biology. However, there will continue to be those who, remembering Kant and seeing clear evidence of systematic regularity as well as variety in the biological realm, feel that Darwin's abandonment of explanation by law and his preoccupation with particulars (adaptation and inheritance), though historically understandable, was nevertheless misguided. For them, Mercer's approach will be unsatisfactory, and they will continue to seek a rational foundation for biology whereby organisms and their evolution become intelligible in terms of universal laws of organization and transformation, not simply in terms of chance events and survival.

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